BORDETELLA PERTUSSIS TOXIN VIRCLIA® IgG MONOTEST

REF VCM092 Σ 24

CE For *in vitro* diagnostic use

INTENDED PURPOSE

Indirect chemiluminescent immunoassay (CLIA) to test IgG antibodies against *Bordetella pertussis* toxin in human serum/plasma.
The test is a quantitative and automated assay, intended to be used as an aid to diagnosis.

INTRODUCTION

Whooping cough is a highly communicable, vaccine-preventable disease that lasts for many weeks, presenting high morbidity and a great mortality in some countries; most deaths occur among unvaccinated children. It is typically manifested in children with paroxysmal spasms of severe coughing, whooping, and post-tussive vomiting. Complications include hypoxia, apnea, pneumonia, seizures, encephalopathy, and malnutrition. Whooping cough is a relatively mild disease in adults. It is caused by the bacterium *Bordetella pertussis*, a small Gram-negative aerobic coccobacillus, which colonizes the cilia of the mammalian respiratory epithelium. Contagion occurs through direct contact with respiratory discharges from infected persons. Specific diagnostic tests include culture, direct immunofluorescence, PCR, and detection of serum antibodies. While culture is almost 100% specific but little sensitive, direct immunofluorescence lacks both sensitivity and specificity.
Serology is widely used for the diagnosis of pertussis in older vaccinated children, adolescents and adults. Yet, the immune responses against infection and vaccination cannot be distinguished.
Due to its sensitivity, specificity, and speed, PCR is accepted as a proof of infection in many countries with notification systems.
Serological diagnosis should only be attempted in patients with symptoms compatible with pertussis. When the clinical course is not typical and prolonged coughing is the only symptom, confirmation of the diagnosis is required. Measurement of IgG-anti-PT is not meaningful in neonates and young infants. Serology can be used for diagnostic purposes only in patients who were not vaccinated during the last twelve months. According to reference laboratories in the EU, ELISA should use purified non-detoxified PT as an antigen, and results should be expressed in International Units per milliliter. Dual sample serology is based on =100% increase in antibody concentration. For single sample serology, different cutoff values have been proposed in various countries. The European Centre for Disease Prevention and Control suggests a dual cut-off between 62 and 125 IU/mL.
Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (ChemiLuminescent ImmunoAssay) tests with shorter incubation times while keeping or improving their sensitivity.

TEST PRINCIPLE

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES

All reagents supplied are ready to use.
Serum dilution solution and conjugate are coloured to help in the performance of the technique.
Sample predilution is not necessary.
Reagents required for the run of the test are included in the monodose presentation.

MATERIALS PROVIDED

[1] VIRCLIA® BORDETELLA PERTUSSIS TOXIN IgG MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:
Wells A, B, C: Reaction wells; wells coated with *Bordetella pertussis* toxin. Contains inactivated antigen. Contains material of animal origin.
Well D: Conjugate: orange; containing anti-human IgG peroxidase conjugate dilution and 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane as preservatives. Contains material of animal origin.
Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers and 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane as preservatives. Contains material of animal origin.
Well F: Calibrator: clear; positive serum dilution containing 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane as preservative. Contains material of human origin. Contains material of animal origin. Equivalent to 120 IU/mL.
Well G: Substrate component B: clear; containing peroxide.
Well H: Substrate component A: clear; containing luminol.
VIRCLIA® BORDETELLA PERTUSSIS TOXIN IgG 5-PL: Labels containing the values obtained for the different parameters during the generation of the master calibration curve at the manufacturer site. It contains specific labels for Vircell's automated systems, which are differentiated by a univocal symbol, as indicated in the section "Symbols used in labels".

Special materials required but not provided:
-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR).
-A CLIA automated processor.

STORAGE AND HANDLING CONDITIONS

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY

VIRCLIA® MONODOSE: Once opened, use it in the same day.
Substrate component A is light sensitive. Avoid light exposure. Substrate solutions should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.
VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
6. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. If the kit or its components are stored in the refrigerator, please bring them at room temperature before use.
11. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
12. Keep containers for samples and reagents closed while they are not being handled.
13. Avoid using samples subjected to repeated freeze-thaw cycles.
14. Handle in aseptic conditions to avoid microbial contaminations.
15. Reagents in this kit could include substances of animal and/or human origin and/or inactivated antigen (refer to "Materials provided"). Although materials of human origin have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, all material and patient specimens should be handled and dispose as potentially infectious using safety laboratory procedures. No present method can offer complete assurance that these or other infectious agents are absent. Dispose of unused reagents and waste in accordance with all applicable regulations.
16. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the VIRCLIA® AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
17. Do not use this product in automated processors unless they have been previously validated for that purpose.
18. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Safety precautions.
Observe the following safety information. For further information a Material Safety Data Sheet is available.

Materials provided	Hazardous ingredients:	Hazard statements (CLP):
[1] VIRCLIA® BORDETELLA PERTUSSIS TOXIN IgG MONODOSE	2-Methyl-2H-isothiazol-3-one CAS-No.: 2682-20-4 EC-No.: 220-239-6	H317 – May cause an allergic skin reaction.

Hazard statements (CLP):	H317 – May cause an allergic skin reaction.
Hazard pictograms (CLP):	 GHS07 Health hazard/ Hazardous to the ozone layer
Signal word (CLP):	Warning
Precautionary statements (CLP):	P261 – Avoid breathing dust/fume/ gas/mist/vapours/spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P302+P352 – If on skin: Wash with plenty of water. P321 – Specific treatment (see supplemental first aid instruction on this label). P333+P313 – If skin irritation or rash occurs: Get medical advice/ attention.

CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum/plasma samples are to be refrigerated (2-8°C) upon collection or frozen (-25- -15°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated samples. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PREPARATORY TREATMENT OF THE DEVICE

All reagents supplied are ready to use.
Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting. Once diluted, store at 2-8°C.

ASSAY PROCEDURE

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

INTERNAL QUALITY CONTROL

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS

Each monodose includes two calibrators (well A and C). They allow the validation of the assay and kit.
The software of the instrument will validate the values obtained for the controls and display them in the results report. Follow the Operator´s Manual of the Automated Processor. Results cannot be validated if the control values deviate from the expected values.

CALCULATIONS AND INTERPRETATION OF RESULTS

In order to estimate the relative concentration of specific anti-*Bordetella pertussis* toxin IgG antibody present in the sample a semilogarithmic graph must be drawn. For example, the logarithm of the IU/mL of each control (120 and 60) and their corresponding RLU should be represented. A straight line should be drawn between the two points that could allow to assign an approximate value of IU/mL to the sample knowing its RLU.
The calibrator has been adjusted to the WHO International Standard Pertussis Antiserum (Human) 1st IS.
Individual laboratories should get their own WHO International Standard to validate the technique.
For single sample serology, different cutoff values have been proposed in various countries. See "Bibliography" for a review.
The European Centre for Disease Prevention and Control suggests a dual cut-off between 62 and 125 IU/mL (1).
Dual sample serology is based on ≥100% increase in antibody concentration (1).

In order to optimize assignment of signals to quantitative values beyond the range defined by the two calibrators, a master calibration curve can be used. For each lot, a 5-PL standard curve is established by Vircell from data generated by running samples containing different concentrations of the standard analyte in repeated independent runs. RLUs of the internal calibrators in each individual strip are used to compensate inter-run and inter-laboratory variations by the use of a master curve factor, calculated from the RLU values of the internal calibrators obtained during the master curve generation.
Determination of antibody concentrations (Conc) is carried out by the 5-parameter logistic (5 PL) model using the following formula:

$$Conc = C\left(\left(\frac{\left(\frac{A}{\left(\frac{MC}{\frac{RLU\ CAL120 + RLU\ CAL60}{2}}\right)}\right) - \left(\frac{D}{\left(\frac{MC}{\frac{RLU\ CAL120 + RLU\ CAL60}{2}}\right)}\right)}{SAMPLE\ RLU - \left(\frac{D}{\left(\frac{MC}{\frac{RLU\ CAL120 + RLU\ CAL60}{2}}\right)}\right)}\right)^{\frac{1}{G}} - 1\right)^{\frac{1}{B}}$$

where MC represents the Master Curve Factor and parameters A, B, C, D and G define the exact shape of the curve:
A. Lower asymptote
B. Slope of the curve
C. Turning point
D. Upper asymptote
G. Parameter for Curve Asymmetry

These variables shown in an external label of the kit must be input onto the software of the instrument to get an automatic calculation if the concentration of the sample derived from this method is intended.

LIMITATIONS OF USE

1. This kit is intended to be used with human serum/plasma.
2. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by direct diagnostic techniques.
3. This test will not indicate the site of infection. It is not intended to replace isolation.
4. Samples collected at the beginning of infection may not have detectable levels of antibodies. In these cases it is recommended to obtain a second sample between 14 and 21 days to be tested in parallel with the original sample, in order to determine a seroconversion.
5. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
6. A negative result in immunosuppressed patients does not always exclude the possibility of infection.
7. Lack of a detectable antibody level does not exclude the possibility of infection.
8. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
9. The performance of this test has not been evaluated for use in patients without clinical signs and symptoms of infection.
10. A calibration curve run in parallel together with the patient samples provides maximum accuracy and minimizes errors derived from interlaboratory or interassay variability.
11. If the master calibration curve is used, the parameters specific for the lot in use must be carefully input in the formula, otherwise the calculated concentrations will be erroneous.
12. The master curve has been adjusted by Vircell for the corresponding automated systems. The parameters that define the curve are different in each system and, therefore, they are not interchangeable between them.

13. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely in low prevalence scenarios.
14. The performance results showed correspond to comparative studies with commercial predicate devices in a defined population sample. Small differences can be found with different populations or different predicate devices.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

Serum/plasma samples were assayed against a commercial ELISA kit or characterized externally. Discordant results were resolved by means of an ELISA assay with the WHO International Standard *Bordetella pertussis* Toxin (LPF) PT 1st IS NIBSC.
The results were as follows:

Samples No.		93
Sensitivity (%)		100
	95% CI	89-100
Specificity (%)		100
	95% CI	94-100
PPV (%)		100
NPV (%)		95
LR+/LR-		-0.92/-0.90

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

WITHIN-RUN PRECISION

3 samples were individually run 10 times each one in a single automated assay in essentially unchanged conditions.
The results were as follows:

Sample	% CV
Sample	8.7
Calibrator 120	2.7
Calibrator 60	5.5

CV: Coefficient of variation

BETWEEN-RUN PRECISION

3 samples were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Sample	% CV
Sample	18.4
Calibrator 120	13.2
Calibrator 60	16.0

CV: Coefficient of variation

INTERFERENCES

Interferences – Endogenous substances

3 samples were tested with each interferent. Specifications were fulfilled in all cases. No interferences were found with haemolytic (8.5 g/L hemoglobin), icteric (6 g/L bilirubin) and hyperlipemic (4 g/L cholesterol and 2 g/L tributyrin) samples.

Interferences – Anticoagulants

3 samples were tested with each anticoagulant. Specifications were fulfilled in all cases. No interferences were found with heparin (30 IU/mL), citrate (0.13 mol/L) and EDTA (2 mg/mL).

CROSS REACTIVITY

5 samples known to be positive for other microorganisms (*Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Legionella pneumophila*, *Coxiella burnetii* and *Clostridium tetani* toxin) were assayed.
No cross-reactivity with *Mycoplasma pneumoniae* (1 sample tested), *Chlamydophila pneumoniae* (1 sample tested), *Legionella pneumophila* (1 sample tested), *Coxiella burnetii* (1 sample tested) and *Clostridium tetani* toxin (1 sample tested) was found.

ANALYTICAL SENSITIVITY / LIMITS OF DETECTION AND QUANTIFICATION (LoB, LoD, LoQ)

4 negative samples were run in triplicate with 2 different batches of the kit during 3 days. LoB, LoD and LoQ were calculated.
The results were as follows:
VIRCLIA® (TB)

LoB	11.79 UI/ml
LoD	16.53 UI/ml
LoQ	35.41 UI/ml

VIRCLIA® LOTUS

LoB	10.40 UI/ml
LoD	12.75 UI/ml
LoQ	22.13 UI/ml

TRUENESS / ACCURACY

This test is only performed when there is a certified reference material or a certified method of reference.
The reference material for this product is the WHO International Standard Pertussis Antiserum (Human) 1st IS.
8 samples were run in triplicate in 3 different runs in, at least, 2 different automated systems. Bias was calculated and the results were as follows:
VIRCLIA® (TB)
Bias (trueness / accuracy) = 11.74 %
VIRCLIA® LOTUS
Bias (trueness / accuracy) = 8.48 %

MEASURING RANGE

Measuring range was stablished as: LoQ + highest internal calibrator.
The results were as follows:
VIRCLIA® (TB)
Measuring range: 370.41 UI/ml
VIRCLIA® LOTUS
Measuring range: 357.13 UI/ml

SYMBOLS USED IN LABELS

IVD	*In vitro* diagnostic medical device
	Use-by (expiry date)
Y°C / X°C	Store at x-y°C
Σ n	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
5PL LOTUS	Parameters of specific Master curve for VIRCLIA® LOTUS automated system
5PL TB	Parameters of specific Master curve for VIRCLIA® (TB) automated system
	Manufacturer

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CHLAMYDOPHILA PNEUMONIAE VIRCLIA® IgG MONOTEST

For *in vitro* diagnostic use

VCM017: Indirect chemiluminescent immunoassay (CLIA) to test IgG antibodies against *Chlamydophila pneumoniae* in human serum/plasma. 24 tests.

INTRODUCTION:

Chlamydia has a great ability to cause respiratory infections, particularly bronchitis and pneumonia. The species most implicated in respiratory infections are *Chlamydophila pneumoniae* and *Chlamydophila psittaci*. The higher incidence takes place in elderly people and it is considered responsible of 10% of all the cases of pneumonia, although it has been considered by some authors as the most frequent cause of those cases of known ethiology. *C. pneumoniae* has been associated with the stablishment of ateromatous disease and heart attach. The seroprevalence to *C. pneumoniae* is low in infants but it can be higher than 50% in adults. In primoinfections IgM antibodies appear before than IgG antibodies while in reinfections IgM is rare, but IgG seroconversion takes place before. COMP (Complexes of Outer Membrane Proteins) antigen is used in the present assay, with LPS removed to avoid cross-reaction with other Chlamydias.

Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (**C**hemi**L**uminescent **I**mmuno**A**ssay) tests with shorter incubation times while keeping or improving their sensitivity.

PRINCIPLE OF THE TEST:

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES:

All reagents supplied are ready to use.

Serum dilution solution and conjugate are coloured to help in the performance of the technique.

Sample predilution is not necessary.

Reagents required for the run of the test are included in the monodose presentation.

KIT CONTENTS:

1 VIRCLIA® CHLAMYDOPHILA PNEUMONIAE IgG MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:

Wells A, B, C: reaction wells; wells coated with purified antigens (Complexes of Outer Membrane Proteins) of *C. pneumoniae*, strain CM-1 (ATCC VR-1360).

Well D: Conjugate: orange; containing anti-human IgG peroxidase conjugate dilution and Neolone and Bronidox as preservatives.

Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers and Neolone and Bronidox as preservatives.

Well F: Calibrator: clear; positive serum dilution containing Neolone and Bronidox as preservative.

Well G: Substrate component B: clear; containing peroxide.

Well H: Substrate component A: clear; containing luminol.

Store at 2-8ºC and check expiration date.

Materials required but not supplied:

-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR)
-Precision micropipettes 5 and 100 µl.
-Eight channel micropipette 100 µl.
-Adapted microplate washer.
-Thermostatized incubator/water bath.
-Microplate luminometer.
-Alternatively, a CLIA automated processor.

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
VIRCLIA® MONODOSE	Once opened, use it in the same day

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.

Do not let the plate dry between washing and reagent addition.

Substrate component A is light sensitive. Avoid light exposure. Substrate solutions should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.

VIRCELL, S.L does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Do not use in the event of damage to the package.
6. Never pipette by mouth.
7. Serum dilution solution, reaction wells, conjugates and calibrator in this kit include substances of animal origin. Calibrator includes as well substances of human origin. Although the human serum controls of this kit have been

tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. Reaction wells are coated with inactivated antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.

8. Do not use this product in automated processors unless they have been previously validated for that purpose.

SPECIMEN COLLECTION AND HANDLING:

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PRELIMINARY PREPARATION OF THE REAGENTS:

All reagents supplied are ready to use.

Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37ºC before diluting. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:

- **AUTOMATED**

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

- **MANUAL**

Contact the manufacturer for further information on the manual procedure.

INTERNAL QUALITY CONTROL:

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.

The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:

Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit.

RLU of the calibrator and the negative control must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	RLU
CALIBRATOR	2-7
NEGATIVE CONTROL	<2

INTERPRETATION OF RESULTS:

Antibody index= (sample RLU/calibrator RLU)

Index	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.

Samples with indexes below 0.9 are considered as not having antibodies of the specificity and class measured by this kit.

Samples with indexes above 1.1 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS:

1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
9. The serologic results must be evaluated in the clinical context of the patient to reach an adecuate diagnosis. In primoinfections by *C. pneumoniae* IgM is generally found, and 3-6 weeks after the onset of the disease high nivels of IgG appear. Otherwise, in reinfections the IgM response is not usually present and there is a quick rise of the levels of IgG and IgA. The seroprevalence to *C. pneumoniae* is high in adults and therefore the presence of antibodies is not indicative of recent infection. Titration of positive samples by microimmunofluorescence can help to confirm the diagnosis. This assay is not designed to measure the immune state to *C. pneumoniae*, but to detect levels of antibodies to the infection.
10. Cross-reaction with *C. psittaci* positive samples have not been tested for this assay due to the low prevalence of the disease and the lack of samples.
11. The performance of this assay has not been evaluated for therapy follow-up.
12. CLIA assays with a single dilution do not present a lineal relation with titers detected by IFA.

13. The performance of the technique for diseases by *C. pneumoniae* other than pneumonia has not been evaluated.
14. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

- **SENSITIVITY AND SPECIFICITY:**

89 serum/plasma samples were assayed against a commercial ELISA kit. The results were as follows:

Samples No.	Sensitivity	Specificity
89	100%	100%

Indeterminate values were omitted from the final calculations.

- **INTRA-ASSAY PRECISION:**

3 sera were individually run 10 times each serum in a single automated assay in essentially unchanged conditions.
The results were as follows:

Serum	N	% C.V.
Sample +	10	6
CAL	10	7
CN	10	10

C.V. Coefficient of variation

- **INTER-ASSAY PRECISION:**

3 sera were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Serum	N	% C.V.
Sample +	10	15
CAL	10	19
CN	10	11

C.V. Coefficient of variation

- **CROSS REACTIVITY AND INTERFERENCES:**

22 samples known to be positive for other bacteria of the syndromic group (*Legionella pneumophila*, *Coxiella burnetii* and *Mycoplasma pneumoniae), Rickettsia conorii* and *Chlamydia trachomatis* were assayed.
The negative results of the test demonstrated the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

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REVISED: 2017/12

CHLAMYDOPHILA PNEUMONIAE VIRCLIA® IgM MONOTEST

For *in vitro* diagnostic use

VCM018: Indirect chemiluminescent immunoassay (CLIA) to test IgM antibodies against *Chlamydophila pneumoniae* in human serum/plasma. 24 tests.

INTRODUCTION:

Chlamydia has a great ability to cause respiratory infections, particularly bronchitis and pneumonia. The species most implicated in respiratory infections are *Chlamydophila pneumoniae* and *Chlamydophila psittaci*. The higher incidence takes place in elderly people and it is considered responsible of 10% of all the cases of pneumonia, although it has been considered by some authors as the most frequent cause of those cases of known ethiology. *C. pneumoniae* has been associated with the stablishment of ateromatous disease and heart attach. The seroprevalence to C. pneumoniae is low in infants but it can be higher than 50% in adults. In primoinfections IgM antibodies appear before than IgG antibodies while in reinfections IgM is rare, but IgG seroconversion takes place before. COMP (Complexes of Outer Membrane Proteins) antigen is used in the present assay, with LPS removed to avoid cross-reaction with other Chlamydias.

Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (**C**hemi**L**uminescent **I**mmuno**A**ssay) tests with shorter incubation times while keeping or improving their sensitivity.

PRINCIPLE OF THE TEST:

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES:

All reagents supplied are ready to use.

Serum dilution solution and conjugate are coloured to help in the performance of the technique.

Sample predilution is not necessary.

Reagents required for the run of the test are included in the monodose presentation.

KIT CONTENTS:

1 VIRCLIA® CHLAMYDOPHILA PNEUMONIAE IgM MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:

Wells A, B, C: reaction wells; wells coated with purified antigens (Complexes of Outer Membrane Proteins) of *C. pneumoniae*, strain CM-1 (ATCC VR-1360).

Well D: Conjugate: orange; containing anti-human IgM peroxidase conjugate dilution and Neolone and Bronidox as preservatives.

Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers, anti-human IgG and Neolone and Bronidox as preservatives.

Well F: Calibrator: clear; positive serum dilution containing Neolone and Bronidox as preservative.

Well G: Substrate component B: clear; containing peroxide.

Well H: Substrate component A: clear; containing luminol.

Store at 2-8ºC and check expiration date.

Materials required but not supplied:

-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR)
-Precision micropipettes 5 and 100 µl.
-Eight channel micropipette 100 µl.
-Adapted microplate washer.
-Thermostatized incubator/water bath.
-Microplate luminometer.
-Alternatively, a CLIA automated processor.

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
VIRCLIA® MONODOSE	Once opened, use it in the same day

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.

Do not let the plate dry between washing and reagent addition.

Substrate component A is light sensitive. Avoid light exposure. Substrate solutions should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.

VIRCELL, S.L does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Do not use in the event of damage to the package.
6. Never pipette by mouth.
7. Serum dilution solution, reaction wells, conjugates and calibrator in this kit include substances of animal origin. Calibrator includes as well substances of human origin. Although the human serum controls of this kit have been

tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. Reaction wells are coated with inactivated antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
8. Do not use this product in automated processors unless they have been previously validated for that purpose.

SPECIMEN COLLECTION AND HANDLING:
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PRELIMINARY PREPARATION OF THE REAGENTS:
All reagents supplied are ready to use.
Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37ºC before diluting. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:
- **AUTOMATED**

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

- **MANUAL**

Contact the manufacturer for further information on the manual procedure.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:
Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit.

RLU of the calibrator and the negative control must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	RLU
CALIBRATOR	2-7
NEGATIVE CONTROL	<2

INTERPRETATION OF RESULTS:
Antibody index= (sample RLU/calibrator RLU)

Index	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 0.9 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 1.1 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS:
1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
9. The serologic results must be evaluated in the clinical context of the patient to reach an adecuate diagnosis. In primoinfections by *C. pneumoniae* IgM is generally found, and 3-6 weeks after the onset of the disease high nivels of IgG appear. Otherwise, in reinfections the IgM response is not usually present and there is a quick rise of the levels of IgG and IgA. The seroprevalence to *C. pneumoniae* is high in adults and therefore the presence of antibodies is not indicative of recent infection. Titration of positive samples by microimmunofluorescence can help to confirm the diagnosis. This assay is not designed to measure the immune state to *C. pneumoniae*, but to detect levels of antibodies to the infection.
10. Cross-reaction with *C. psittaci* positive samples have not been tested for this assay due to the low prevalence of the disease and the lack of samples.
11. The performance of this assay has not been evaluated for therapy follow-up.
12. CLIA assays with a single dilution do not present a lineal relation with titers detected by IFA.

13. The performance of the technique for diseases by *C. pneumoniae* other than pneumonia has not been evaluated.
14. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

- **SENSITIVITY AND SPECIFICITY:**

94 serum/plasma samples were assayed against a commercial ELISA kit. The results were as follows:

Samples No.	Sensitivity	Specificity
94	100%	98%

Indeterminate values were omitted from the final calculations.

- **INTRA-ASSAY PRECISION:**

3 sera were individually run 10 times each serum in a single automated assay in essentially unchanged conditions.
The results were as follows:

Serum	N	% C.V.
Sample +	10	8
CAL	10	8
CN	10	10

C.V. Coefficient of variation

- **INTER-ASSAY PRECISION:**

3 sera were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Serum	N	% C.V.
Sample +	10	16
CAL	10	12
CN	10	19

C.V. Coefficient of variation

- **CROSS REACTIVITY AND INTERFERENCES:**

12 serum samples known to be positive for other bacterial members of the syndromic group (Legionella pneumophila, Coxiella burnetii and Mycoplasma pneumoniae) and *Rickettsia conorii* and *Chlamydia trachomatis* were assayed. 2 samples known to be positive for rheumatoid factor were assayed.
The negative results of the test demonstrated the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

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REVISED: 2017/12

HERPES SIMPLEX 1+2 VIRCLIA® IgG MONOTEST

For *in vitro* diagnostic use

VCM034: Indirect chemiluminescent immunoassay (CLIA) to test IgG antibodies against herpes simplex type 1+ 2 in human serum/plasma. 24 tests.

INTRODUCTION:

Human infections with herpes simplex virus (HSV) are ubiquitous throughout the world. Primary infection is subclinical in a majority of cases. The most frequent manifestation of primary HSV-1 is pharyngitis and gingivostomatitis but other typical manifestations are conjunctivitis, keratitis, vesicular eruptions of skin, and encephalitis. HSV-2 is the most frequent agent of genital ulcera in the Western world and it can produce aseptic meningitis and neonatal herpes infection. The most used serological methods are complement fixation reaction, neutralization and enzyme-linked immunosorbent assay (ELISA). The two HSV types share many common epitopes that give rise to strongly cross-reacting antigens; therefore, it is difficult to achieve the serological differentiation. The immune response is stronger in the primary infection than during relapses.

Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (**C**hemi**L**uminescent **I**mmuno**A**ssay) tests with shorter incubation times while keeping or improving their sensitivity.

PRINCIPLE OF THE TEST:

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES:

All reagents supplied are ready to use.

Serum dilution solution and conjugate are coloured to help in the performance of the technique.

Sample predilution is not necessary.

Reagents required for the run of the test are included in the monodose presentation.

KIT CONTENTS:

1 VIRCLIA® HERPES SIMPLEX 1+2 IgG MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:

Wells A, B, C: reaction wells; wells coated with common antigens of herpes simplex (HSV) type 1 and 2, strain HSV-1: MacIntyre (ATCC VR-539) and HSV-2: MS (ATCC VR-540).

Well D: Conjugate: orange; containing anti-human IgG peroxidase conjugate dilution and Neolone and Bronidox as preservatives.

Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers and Neolone and Bronidox as preservatives.

Well F: Calibrator: clear; positive serum dilution containing Neolone and Bronidox as preservative.

Well G: Substrate component B: clear; containing peroxide.

Well H: Substrate component A: clear; containing luminol.

Store at 2-8ºC and check expiration date.

Materials required but not supplied:

-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR)

-Precision micropipettes 5 and 100 µl.

-Eight channel micropipette 100 µl.

-Adapted microplate washer.

-Thermostatized incubator/water bath.

-Microplate luminometer.

-Alternatively, a CLIA automated processor.

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
VIRCLIA® MONODOSE	Once opened, use it in the same day

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.

Do not let the plate dry between washing and reagent addition.

Substrate component A is light sensitive. Avoid light exposure.

Substrate solution should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.

VIRCELL, S.L does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Do not use in the event of damage to the package.
6. Never pipette by mouth.
7. Serum dilution solution, reaction wells, conjugates and calibrator in this kit include substances of animal origin. Calibrator includes as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. Reaction wells are coated

with inactivated antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
8. Substrate solution may be irritant to eyes, respiratory system and skin. In case of contact with this solution, rinse thoroughly with water and seek medical attention. For further information a Material Safety Data Sheet is available.
9. Do not use this product in automated processors unless they have been previously validated for that purpose.

SPECIMEN COLLECTION AND HANDLING:
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PRELIMINARY PREPARATION OF THE REAGENTS:
All reagents supplied are ready to use.
Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37ºC before diluting. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:
- **AUTOMATED**

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

- **MANUAL**

1. Set incubator/water bath to 37±1ºC.
2. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
3. Remove the monodoses from the package and let them reach room temperature before use (approximately 20 minutes). Determine the numbers of monodoses to be employed (one for every sample to be tested).
4. Place the strips into a frame. With the aid of a clean pipette tip, puncture the foil of the well in position E (see the left side of the frame for reference), draw 100 µl of reagent (sample diluent) and dispense it into the white well in position B. Add 5 µl of sample into the white well in position B. Mix homogenously with the aid of a pipette. With the aid of a clean pipette tip, puncture the foil of the well in position F, draw 100 µl of reagent (calibrator) and dispense it into white well in position A. Draw 80 µl of reagent from well E with a new clean tip and add it into the white well in position C, then draw 20 µl of reagent from well F and add it into the white well in position C. Mix homogenously with the aid of a pipette.
5. Incubate at 37±1ºC for 20 minutes.
6. Aspirate liquid from wells A, B, C and wash five times with 0.3 ml of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) per well. Drain off any remaining liquid.
7. Immediately add 50 µl of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) into each one of wells A, B, C.
8. With the aid of a clean pipette tip, puncture the foil of the well in position D, draw 50 µl of reagent (conjugate) and dispense it into the white well in position A. Repeat this step for wells B and C.
9. Incubate at 37±1ºC for 20 minutes.
10. Aspirate liquid from wells A, B, C and wash five times with 0.3 ml of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) per well. Drain off any remaining liquid.
11. With the aid of a clean pipette tip, puncture the foil of the well in position H, draw 50 µl of reagent (substrate component A) and dispense it into the white well in position A. Repeat this step for wells B and C.
12. With the aid of a clean pipette tip, puncture the foil of the well in position G, draw 50 µl of reagent (substrate component B) and dispense it into the white well in position A. Repeat this step for wells B and C. Mix gently for 10 seconds with the aid of a microtiter plate shaker or by gently tapping on the sides of the plate.
13. Incubate at 37ºC for 5 minutes protected from light.
14. Measure relative luminescence units (RLU) in wells A, B, C with the help of a luminometer.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:
Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit.

RLU of the calibrator and the negative control must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	RLU
CALIBRATOR	2-7
NEGATIVE CONTROL	<2

INTERPRETATION OF RESULTS:
Antibody index= (sample RLU/calibrator RLU)

Index	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 0.9 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 1.1 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS:

1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
9. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

- **SENSITIVITY AND SPECIFICITY:**

53 serum/plasma samples were assayed against a commercial ELISA kit. The results were as follows:

Samples No.	Sensitivity	Specificity
53	97%	100%

Indeterminate values were omitted from the final calculations.

- **INTRA-ASSAY PRECISION:**

3 sera were individually run 10 times each serum in a single automated assay in essentially unchanged conditions.
The results were as follows:

Serum	N	% C.V.
Sample +	10	6
CAL	10	6
CN	10	8

C.V. Coefficient of variation

- **INTER-ASSAY PRECISION:**

3 sera were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Serum	N	% C.V.
Sample +	10	9
CAL	10	16
CN	10	17

C.V. Coefficient of variation

- **CROSS REACTIVITY AND INTERFERENCES:**

7 samples known to be positive for other herpesvirus (varicella-zoster, Esptein-Barr virus and cytomegalovirus) were assayed. 3 samples known to be positive for antinuclear antibodies were assayed.
The negative results of the test demonstrated the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

BIBLIOGRAPHY:

1. Arvaja, M., M. Lehtinen, P. Koskela, M. Lappalainen, J. Paavonen, and T. Vesikari. 1999. Serological evaluation of herpes simplex virus type 1 and type 2 infections in pregnancy. Sex Transm Infect 75:168-71.
2. Ashley, R. L., J. Militoni, F. Lee, A. Nahmias, and L. Corey. 1988. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. J Clin Microbiol 26:662-7.
3. Chonmaitree, T., C. D. Baldwin, and H. L. Lucia. 1989. Role of the virology laboratory in diagnosis and management of patients with central nervous system disease. Clin Microbiol Rev 2:1-14.
4. Debyser, Z., M. Reynders, P. Goubau, and J. Desmyter. 1997. Comparative evaluation of three ELISA techniques and an indirect immunofluorescence assay for the serological diagnosis of Epstein-Barr virus infection. Clin Diagn Virol 8:71-81.
5. Hampar, B., M. Zweig, S. D. Showalter, S. V. Bladen, and C. W. Riggs. 1985. Enzyme-linked immunosorbent assay for determination of antibodies against herpes simplex virus types 1 and 2 in human sera. J Clin Microbiol 21:496-500.
6. Katz, D., J. K. Hilliard, R. R. Mirkovic, and R. A. Word. 1986. ELISA for detection of IgG and IgM antibodies to HSV-1 and HSV-2 in human sera. J Virol Methods 14:43-55.
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8. Sharief, M. K. and E. J. Thompson. 1990. A sensitive ELISA system for the rapid detection of virus specific IgM antibodies in the cerebrospinal fluid. J Immunol Methods 130:19-24.
9. Velan, B., M. Halmann. 1978. Chemiluminescence immunoassay. A new sensitive method for determination of antigens. Immunochemistry. 15: 331–333.
10. Whitehead, T.P., L.J. Kricka, T.J. Carter, G.H. Thorpe. 1979. Analytical luminescence: its potential in the clinical laboratory. Clin Chem. 25:1531-46.
11. Zhao, L., L. Sun, X. Chu. 2009. Chemiluminescence immunoassay. TrAC-Trend Anal Chem. 28: 404–415.

For any questions please contact:
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REVISED: 06/2014

HERPES SIMPLEX 1+2 VIRCLIA® IgM MONOTEST

For *in vitro* diagnostic use

VCM036: Indirect chemiluminescent immunoassay (CLIA) to test IgM antibodies against herpes simplex type 1+ 2 in human serum/plasma. 24 tests.

INTRODUCTION:

Human infections with herpes simplex virus (HSV) are ubiquitous throughout the world. Primary infection is subclinical in a majority of cases. The most frequent manifestation of primary HSV-1 is pharyngitis and gingivostomatitis but other typical manifestations are conjunctivitis, keratitis, vesicular eruptions of skin, and encephalitis. HSV-2 is the most frequent agent of genital ulcera in the Western world and it can produce aseptic meningitis and neonatal herpes infection. The most used serological methods are complement fixation reaction, neutralization and enzyme-linked immunosorbent assay (ELISA). The two HSV types share many common epitopes that give rise to strongly cross-reacting antigens; therefore, it is difficult to achieve the serological differentiation. The immune response is stronger in the primary infection than during relapses.

Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (**C**hemi**L**uminescent **I**mmuno**A**ssay) tests with shorter incubation times while keeping or improving their sensitivity.

PRINCIPLE OF THE TEST:

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES:

All reagents supplied are ready to use.

Serum dilution solution and conjugate are coloured to help in the performance of the technique.

Sample predilution is not necessary.

Reagents required for the run of the test are included in the monodose presentation.

KIT CONTENTS:

1 VIRCLIA® HERPES SIMPLEX 1+2 IgM MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:

Wells A, B, C: reaction wells; wells coated with common antigens of herpes simplex (HSV) type 1 and 2, strain HSV-1: MacIntyre (ATCC VR-539) and HSV-2: MS (ATCC VR-540).

Well D: Conjugate: orange; containing anti-human IgM peroxidase conjugate dilution and Neolone and Bronidox as preservatives.

Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers, anti-human IgG and Neolone and Bronidox as preservatives.

Well F: Calibrator: clear; positive serum dilution containing Neolone and Bronidox as preservative.

Well G: Substrate component B: clear; containing peroxide.

Well H: Substrate component A: clear; containing luminol.

Store at 2-8ºC and check expiration date.

Materials required but not supplied:

-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR)

-Precision micropipettes 5 and 100 µl.

-Eight channel micropipette 100 µl.

-Adapted microplate washer.

-Thermostatized incubator/water bath.

-Microplate luminometer.

-Alternatively, a CLIA automated processor.

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
VIRCLIA® MONODOSE	Once opened, use it in the same day

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.

Do not let the plate dry between washing and reagent addition.

Substrate component A is light sensitive. Avoid light exposure.

Substrate solution should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.

VIRCELL, S.L does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Do not use in the event of damage to the package.
6. Never pipette by mouth.
7. Serum dilution solution, reaction wells, conjugates and calibrator in this kit include substances of animal origin. Calibrator includes as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be handled as potentially infectious. Reaction wells are coated

with inactivated antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
8. Substrate solution may be irritant to eyes, respiratory system and skin. In case of contact with this solution, rinse thoroughly with water and seek medical attention. For further information a Material Safety Data Sheet is available.
9. Do not use this product in automated processors unless they have been previously validated for that purpose.

SPECIMEN COLLECTION AND HANDLING:
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PRELIMINARY PREPARATION OF THE REAGENTS:
All reagents supplied are ready to use.
Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37ºC before diluting. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:
- **AUTOMATED**

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

- **MANUAL**

1. Set incubator/water bath to 37±1ºC.
2. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
3. Remove the monodoses from the package and let them reach room temperature before use (approximately 20 minutes). Determine the numbers of monodoses to be employed (one for every sample to be tested).
4. Place the strips into a frame. With the aid of a clean pipette tip, puncture the foil of the well in position E (see the left side of the frame for reference), draw 100 µl of reagent (sample diluent) and dispense it into the white well in position B. Add 5 µl of sample into the white well in position B. Mix homogenously with the aid of a pipette. With the aid of a clean pipette tip, puncture the foil of the well in position F, draw 100 µl of reagent (calibrator) and dispense it into white well in position A. Draw 80 µl of reagent from well E with a new clean tip and add it into the white well in position C, then draw 20 µl of reagent from well F and add it into the white well in position C. Mix homogenously with the aid of a pipette.
5. Incubate at 37±1ºC for 20 minutes.
6. Aspirate liquid from wells A, B, C and wash five times with 0.3 ml of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) per well. Drain off any remaining liquid.
7. Immediately add 50 µl of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) into each one of wells A, B, C.
8. With the aid of a clean pipette tip, puncture the foil of the well in position D, draw 50 µl of reagent (conjugate) and dispense it into the white well in position A. Repeat this step for wells B and C.
9. Incubate at 37±1ºC for 20 minutes.
10. Aspirate liquid from wells A, B, C and wash five times with 0.3 ml of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) per well. Drain off any remaining liquid.
11. With the aid of a clean pipette tip, puncture the foil of the well in position H, draw 50 µl of reagent (substrate component A) and dispense it into the white well in position A. Repeat this step for wells B and C.
12. With the aid of a clean pipette tip, puncture the foil of the well in position G, draw 50 µl of reagent (substrate component B) and dispense it into the white well in position A. Repeat this step for wells B and C. Mix gently for 10 seconds with the aid of a microtiter plate shaker or by gently tapping on the sides of the plate.
13. Incubate at 37ºC for 5 minutes protected from light.
14. Measure relative luminescence units (RLU) in wells A, B, C with the help of a luminometer.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:
Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit.

RLU of the calibrator and the negative control must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	RLU
CALIBRATOR	2-7
NEGATIVE CONTROL	<2

INTERPRETATION OF RESULTS:
Antibody index= (sample RLU/calibrator RLU)

Index	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 0.9 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 1.1 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS:

1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
9. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

- **SENSITIVITY AND SPECIFICITY:**

55 serum/plasma samples were assayed against a commercial ELISA kit. The results were as follows:

Samples No.	Sensitivity	Specificity
55	100%	100%

Indeterminate values were omitted from the final calculations.

- **INTRA-ASSAY PRECISION:**

3 sera were individually run 10 times each serum in a single automated assay in essentially unchanged conditions.
The results were as follows:

Serum	N	% C.V.
Sample +	10	7
CAL	10	7
CN	10	9

C.V. Coefficient of variation

- **INTER-ASSAY PRECISION:**

3 sera were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Serum	N	% C.V.
Sample +	10	6
CAL	10	8
CN	10	18

C.V. Coefficient of variation

- **CROSS REACTIVITY AND INTERFERENCES:**

7 samples known to be positive for other herpesvirus (varicella-zoster, Esptein-Barr virus and cytomegalovirus) were assayed. 3 samples known to be positive for rheumatoid factor were assayed. The negative results of the test demonstrated the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

BIBLIOGRAPHY:

1. Arvaja, M., M. Lehtinen, P. Koskela, M. Lappalainen, J. Paavonen, and T. Vesikari. 1999. Serological evaluation of herpes simplex virus type 1 and type 2 infections in pregnancy. Sex Transm Infect 75:168-71.
2. Ashley, R. L., J. Militoni, F. Lee, A. Nahmias, and L. Corey. 1988. Comparison of Western blot (immunoblot) and glycoprotein G-specific immunodot enzyme assay for detecting antibodies to herpes simplex virus types 1 and 2 in human sera. J Clin Microbiol 26:662-7.
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4. Debyser, Z., M. Reynders, P. Goubau, and J. Desmyter. 1997. Comparative evaluation of three ELISA techniques and an indirect immunofluorescence assay for the serological diagnosis of Epstein-Barr virus infection. Clin Diagn Virol 8:71-81.
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8. Sharief, M. K. and E. J. Thompson. 1990. A sensitive ELISA system for the rapid detection of virus specific IgM antibodies in the cerebrospinal fluid. J Immunol Methods 130:19-24.
9. Velan, B., M. Halmann. 1978. Chemiluminescence immunoassay. A new sensitive method for determination of antigens. Immunochemistry. 15: 331–333.
10. Whitehead, T.P., L.J. Kricka, T.J. Carter, G.H. Thorpe. 1979. Analytical luminescence: its potential in the clinical laboratory. Clin Chem. 25:1531-46.
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For any questions please contact:
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REVISED: 06/2014

MYCOPLASMA PNEUMONIAE VIRCLIA® IgG MONOTEST

For *in vitro* diagnostic use

VCM062: Indirect chemiluminescent immunoassay (CLIA) to test IgG antibodies against *Mycoplasma pneumoniae* in human serum/plasma. 24 tests.

INTRODUCTION:

Atypical pneumonia produced by *M. pneumoniae* is most frequently found in children and adolescents. The isolation in culture is tedious and consequently serological diagnosis is most frequently performed. IgM can be detected a week after the appearance of symptoms and IgG a week later. IgM does not appear in all patients and very rarely in reinfections. Thus, 56% of patients over 40 show no IgM response. In children and adolescents, IgM detection is more suitable. IgM is a good marker of acute illness because it persists for only 3-4 months, while IgG antibodies are found during years after infection. The most traditional serological method has been the complement fixation reaction whereas enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA) are now more often used.

Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (**C**hemi**L**uminescent **I**mmuno**A**ssay) tests with shorter incubation times while keeping or improving their sensitivity.

PRINCIPLE OF THE TEST:

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES:

All reagents supplied are ready to use.
Serum dilution solution and conjugate are coloured to help in the performance of the technique.
Sample predilution is not necessary.
Reagents required for the run of the test are included in the monodose presentation.

KIT CONTENTS:

1 VIRCLIA® MYCOPLASMA PNEUMONIAE IgG MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:

Wells A, B, C: reaction wells; wells coated with detergent-soluble antigens (containing P1 protein) of *M. pneumoniae*, strain FH (ATCC 15531).

Well D: Conjugate: orange; containing anti-human IgG peroxidase conjugate dilution and Neolone and Bronidox as preservatives.

Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers and Neolone and Bronidox as preservatives.

Well F: Calibrator: clear; positive serum dilution containing Neolone and Bronidox as preservative.

Well G: Substrate component B: clear; containing peroxide.

Well H: Substrate component A: clear; containing luminol.

Store at 2-8ºC and check expiration date.

Materials required but not supplied:

-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR)
-Precision micropipettes 5 and 100 µl.
-Eight channel micropipette 100 µl.
-Adapted microplate washer.
-Thermostatized incubator/water bath.
-Microplate luminometer.
-Alternatively, a CLIA automated processor.

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
VIRCLIA® MONODOSE	Once opened, use it in the same day

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.
Do not let the plate dry between washing and reagent addition.
Substrate component A is light sensitive. Avoid light exposure. Substrate solution should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.

VIRCELL, S.L does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Do not use in the event of damage to the package.
6. Never pipette by mouth.
7. Serum dilution solution, reaction wells, conjugates and calibrator in this kit include substances of animal origin. Calibrator includes as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be

handled as potentially infectious. Reaction wells are coated with inactivated antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
8. Substrate solution may be irritant to eyes, respiratory system and skin. In case of contact with this solution, rinse thoroughly with water and seek medical attention. For further information a Material Safety Data Sheet is available.
9. Do not use this product in automated processors unless they have been previously validated for that purpose.

SPECIMEN COLLECTION AND HANDLING:
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PRELIMINARY PREPARATION OF THE REAGENTS:
All reagents supplied are ready to use.
Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37ºC before diluting. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:
- **AUTOMATED**

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

- **MANUAL**

1. Set incubator/water bath to 37±1ºC.
2. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
3. Remove the monodoses from the package and let them reach room temperature before use (approximately 20 minutes). Determine the numbers of monodoses to be employed (one for every sample to be tested).
4. Place the strips into a frame. With the aid of a clean pipette tip, puncture the foil of the well in position E (see the left side of the frame for reference), draw 100 µl of reagent (sample diluent) and dispense it into the white well in position B. Add 5 µl of sample into the white well in position B. Mix homogenously with the aid of a pipette. With the aid of a clean pipette tip, puncture the foil of the well in position F, draw 100 µl of reagent (calibrator) and dispense it into white well in position A. Draw 80 µl of reagent from well E with a new clean tip and add it into the white well in position C, then draw 20 µl of reagent from well F and add it into the white well in position C. Mix homogenously with the aid of a pipette.
5. Incubate at 37±1ºC for 20 minutes.
6. Aspirate liquid from wells A, B, C and wash five times with 0.3 ml of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) per well. Drain off any remaining liquid.
7. Immediately add 50 µl of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) into each one of wells A, B, C.
8. With the aid of a clean pipette tip, puncture the foil of the well in position D, draw 50 µl of reagent (conjugate) and dispense it into the white well in position A. Repeat this step for wells B and C.
9. Incubate at 37±1ºC for 20 minutes.
10. Aspirate liquid from wells A, B, C and wash five times with 0.3 ml of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) per well. Drain off any remaining liquid.
11. With the aid of a clean pipette tip, puncture the foil of the well in position H, draw 50 µl of reagent (substrate component A) and dispense it into the white well in position A. Repeat this step for wells B and C.
12. With the aid of a clean pipette tip, puncture the foil of the well in position G, draw 50 µl of reagent (substrate component B) and dispense it into the white well in position A. Repeat this step for wells B and C. Mix gently for 10 seconds with the aid of a microtiter plate shaker or by gently tapping on the sides of the plate.
13. Incubate at 37ºC for 5 minutes protected from light.
14. Measure relative luminescence units (RLU) in wells A, B, C with the help of a luminometer.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:
Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit.

RLU of the calibrator and the negative control must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	RLU
CALIBRATOR	2-7
NEGATIVE CONTROL	<2

INTERPRETATION OF RESULTS:
Antibody index= (sample RLU/calibrator RLU)

Index	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 0.9 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 1.1 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS:

1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
9. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

- **SENSITIVITY AND SPECIFICITY:**

91 serum/plasma samples were assayed against a commercial ELISA kit. The results were as follows:

Samples No.	Sensitivity	Specificity
91	98%	100%

Indeterminate values were omitted from the final calculations.

- **INTRA-ASSAY PRECISION:**

3 sera were individually run 10 times each serum in a single automated assay in essentially unchanged conditions.
The results were as follows:

Serum	N	% C.V.
Sample +	10	8
CAL	10	7
CN	10	7

C.V. Coefficient of variation

- **INTER-ASSAY PRECISION:**

3 sera were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Serum	N	% C.V.
Sample +	10	19
CAL	10	19
CN	10	16

C.V. Coefficient of variation

- **CROSS REACTIVITY AND INTERFERENCES:**

6 samples known to be positive for other bacteria of the syndromic group (*Legionella pneumophila*, *Chlamydophila pneumoniae* and *Coxiella burnetii*) were assayed.
The negative results of the test demonstrated the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

BIBLIOGRAPHY:

1. Aubert, G., B. Pozzetto, O. G. Gaudin, J. Hafid, A. D. Mbida, and A. Ros. 1992. Evaluation of five commercial tests: complement fixation, microparticle agglutination, indirect immunofluorescence, enzyme-linked immunosorbent assay and latex agglutination, in comparison to immunoblotting for *Mycoplasma pneumoniae* serology. Ann Biol Clin (Paris) 50:593-7.
2. Busolo, F., E. Tonin, and G. A. Meloni. 1983. Enzyme-linked immunosorbent assay for serodiagnosis of *Mycoplasma pneumoniae* infections. J Clin Microbiol 18:432-5.
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4. Dussaix, E., A. Slim, and P. Tournier. 1983. Comparison of enzyme-linked immunosorbent assay (ELISA) and complement fixation test for detection of *Mycoplasma pneumoniae* antibodies. J Clin Pathol 36:228-32.
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8. Thacker, W. L. and D. F. Talkington. 2000. Analysis of complement fixation and commercial enzyme immunoassays for detection of antibodies to *Mycoplasma pneumoniae* in human serum. Clin Diagn Lab Immunol 7:778-80.

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10. Wreghitt, T. G. and M. Sillis. 1985. A micro-capture ELISA for detecting *Mycoplasma pneumoniae* IgM: comparison with indirect immunofluorescence and indirect ELISA. J Hyg (Lond) 94:217-27.
11. Velan, B., M. Halmann. 1978. Chemiluminescence immunoassay. A new sensitive method for determination of antigens. Immunochemistry. 15: 331–333.
12. Whitehead, T.P., L.J. Kricka, T.J. Carter, G.H. Thorpe. 1979. Analytical luminescence: its potential in the clinical laboratory. Clin Chem. 25:1531-46.
13. Zhao, L., L. Sun, X. Chu. 2009. Chemiluminescence immunoassay. TrAC-Trend Anal Chem. 28: 404–415.

For any questions please contact:
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REVISED: 06/2014

MYCOPLASMA PNEUMONIAE VIRCLIA® IgM MONOTEST

For *in vitro* diagnostic use

VCM064: Indirect chemiluminescent immunoassay (CLIA) to test IgM antibodies against *Mycoplasma pneumoniae* in human serum/plasma. 24 tests.

INTRODUCTION:

Atypical pneumonia produced by *M. pneumoniae* is most frequently found in children and adolescents. The isolation in culture is tedious and consequently serological diagnosis is most frequently performed. IgM can be detected a week after the appearance of symptoms and IgG a week later. IgM does not appear in all patients and very rarely in reinfections. Thus, 56% of patients over 40 show no IgM response. In children and adolescents, IgM detection is more suitable. IgM is a good marker of acute illness because it persists for only 3-4 months, while IgG antibodies are found during years after infection. The most traditional serological method has been the complement fixation reaction whereas enzyme-linked immunosorbent assay (ELISA) and immunofluorescence assay (IFA) are now more often used.

Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (**C**hemi**L**uminescent **I**mmuno**A**ssay) tests with shorter incubation times while keeping or improving their sensitivity.

PRINCIPLE OF THE TEST:

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES:

All reagents supplied are ready to use.
Serum dilution solution and conjugate are coloured to help in the performance of the technique.
Sample predilution is not necessary.
Reagents required for the run of the test are included in the monodose presentation.

KIT CONTENTS:

1 VIRCLIA® MYCOPLASMA PNEUMONIAE IgM MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:

Wells A, B, C: reaction wells; wells coated with detergent-soluble antigens (containing P1 protein) of *M. pneumoniae*, strain FH (ATCC 15531).

Well D: Conjugate: orange; containing anti-human IgM peroxidase conjugate dilution and Neolone and Bronidox as preservatives.

Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers, anti-human IgG and Neolone and Bronidox as preservatives.

Well F: Calibrator: clear; positive serum dilution containing Neolone and Bronidox as preservative.

Well G: Substrate component B: clear; containing peroxide.

Well H: Substrate component A: clear; containing luminol.

Store at 2-8ºC and check expiration date.

Materials required but not supplied:

-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR)
-Precision micropipettes 5 and 100 µl.
-Eight channel micropipette 100 µl.
-Adapted microplate washer.
-Thermostatized incubator/water bath.
-Microplate luminometer.
-Alternatively, a CLIA automated processor.

STORAGE REQUIREMENTS:

Store at 2-8ºC. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8ºC.

STORAGE OF REAGENTS ONCE OPENED:

Reagent	Stability
VIRCLIA® MONODOSE	Once opened, use it in the same day

STABILITY AND HANDLING OF REAGENTS:

Handle reagents in aseptic conditions to avoid microbial contaminations.
Do not let the plate dry between washing and reagent addition.
Substrate component A is light sensitive. Avoid light exposure. Substrate solution should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.

VIRCELL, S.L does not accept responsibility for the mishandling of the reagents included in the kit.

RECOMMENDATIONS AND PRECAUTIONS:

1. For *in vitro* diagnosis use only. For professional use only.
2. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
3. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
4. Wear protective disposable gloves, laboratory coats and eye protection when handling specimens. Wash hands thoroughly after manipulating samples. Besides, follow all safety protocols in use in your laboratory.
5. Do not use in the event of damage to the package.
6. Never pipette by mouth.
7. Serum dilution solution, reaction wells, conjugates and calibrator in this kit include substances of animal origin. Calibrator includes as well substances of human origin. Although the human serum controls of this kit have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, control sera and patient specimens should be

handled as potentially infectious. Reaction wells are coated with inactivated antigen. Nevertheless, they should be considered potentially infectious and handled with care. No present method can offer complete assurance that infectious agents are absent. All material should be handled and disposed as potentially infectious. Observe the local regulations for clinical waste disposal.
8. Substrate solution may be irritant to eyes, respiratory system and skin. In case of contact with this solution, rinse thoroughly with water and seek medical attention. For further information a Material Safety Data Sheet is available.
9. Do not use this product in automated processors unless they have been previously validated for that purpose.

SPECIMEN COLLECTION AND HANDLING:
Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum samples are to be refrigerated (2-8ºC) upon collection or frozen (-20ºC) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated sera. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PRELIMINARY PREPARATION OF THE REAGENTS:
All reagents supplied are ready to use.
Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37ºC before diluting. Once diluted, store at 2-8ºC.

ASSAY PROCEDURE:
- **AUTOMATED**

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

- **MANUAL**

1. Set incubator/water bath to 37±1ºC.
2. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
3. Remove the monodoses from the package and let them reach room temperature before use (approximately 20 minutes). Determine the numbers of monodoses to be employed (one for every sample to be tested).
4. Place the strips into a frame. With the aid of a clean pipette tip, puncture the foil of the well in position E (see the left side of the frame for reference), draw 100 µl of reagent (sample diluent) and dispense it into the white well in position B. Add 5 µl of sample into the white well in position B. Mix homogenously with the aid of a pipette. With the aid of a clean pipette tip, puncture the foil of the well in position F, draw 100 µl of reagent (calibrator) and dispense it into white well in position A. Draw 80 µl of reagent from well E with a new clean tip and add it into the white well in position C, then draw 20 µl of reagent from well F and add it into the white well in position C. Mix homogenously with the aid of a pipette.
5. Incubate at 37±1ºC for 20 minutes.
6. Aspirate liquid from wells A, B, C and wash five times with 0.3 ml of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) per well. Drain off any remaining liquid.
7. Immediately add 50 µl of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) into each one of wells A, B, C.
8. With the aid of a clean pipette tip, puncture the foil of the well in position D, draw 50 µl of reagent (conjugate) and dispense it into the white well in position A. Repeat this step for wells B and C.
9. Incubate at 37±1ºC for 20 minutes.
10. Aspirate liquid from wells A, B, C and wash five times with 0.3 ml of VIRCLIA® WASHING SOLUTION (diluted according to the instructions) per well. Drain off any remaining liquid.
11. With the aid of a clean pipette tip, puncture the foil of the well in position H, draw 50 µl of reagent (substrate component A) and dispense it into the white well in position A. Repeat this step for wells B and C.
12. With the aid of a clean pipette tip, puncture the foil of the well in position G, draw 50 µl of reagent (substrate component B) and dispense it into the white well in position A. Repeat this step for wells B and C. Mix gently for 10 seconds with the aid of a microtiter plate shaker or by gently tapping on the sides of the plate.
13. Incubate at 37ºC for 5 minutes protected from light.
14. Measure relative luminescence units (RLU) in wells A, B, C with the help of a luminometer.

INTERNAL QUALITY CONTROL:
Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS:
Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit.

RLU of the calibrator and the negative control must fall in the following ranges. Otherwise, the test is invalid and must be repeated.

Control	RLU
CALIBRATOR	2-7
NEGATIVE CONTROL	<2

INTERPRETATION OF RESULTS:
Antibody index= (sample RLU/calibrator RLU)

Index	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 0.9 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 1.1 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS:

1. This kit is intended to be used with human serum/plasma.
2. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results. In particular, correct sample and reagent pipetting, along with careful washing and timing of the incubation steps are essential for accurate results.
3. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by isolation techniques.
4. This test will not indicate the site of infection. It is not intended to replace isolation.
5. Lack of significant rise in antibody level does not exclude the possibility of infection.
6. Samples collected very early in the course of an infection may not have detectable levels of IgG. In such cases, it is recommended an IgM assay be performed or a second serum sample be obtained 14 to 21 days later to be tested in parallel with the original sample to determine seroconversion.
7. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
8. The results of a single-specimen antibody determination should not be used to aid in the diagnosis of recent infection. Paired samples (acute and convalescent) should be collected and tested concurrently to look for seroconversion or a significant rise in antibody level.
9. IgM does not appear in all patients and very rarely in reinfections. Thus, 56% of patients over 40 show no IgM response. In children and adolescents, IgM detection is more suitable. IgM is a good marker of acute illness because it persists for only 3-4 months, while IgG antibodies are found during years after infection.
10. The performance results showed correspond to comparative studies with commercial predicative devices in a defined population sample. Small differences can be found with different populations or different predicative devices.

PERFORMANCES:

- **SENSITIVITY AND SPECIFICITY:**

74 serum/plasma samples were assayed against a commercial ELISA kit. The results were as follows:

Samples No.	Sensitivity	Specificity
74	100%	98%

Indeterminate values were omitted from the final calculations.

- **INTRA-ASSAY PRECISION:**

3 sera were individually run 10 times each serum in a single automated assay in essentially unchanged conditions.
The results were as follows:

Serum	N	% C.V.
Sample +	10	8
CAL	10	9
CN	10	9

C.V. Coefficient of variation

- **INTER-ASSAY PRECISION:**

3 sera were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Serum	N	% C.V.
Sample +	10	17
CAL	10	11
CN	10	18

C.V. Coefficient of variation

- **CROSS REACTIVITY AND INTERFERENCES:**

6 samples known to be positive for other bacteria of the syndromic group (*Legionella pneumophila*, *Chlamydophila pneumoniae* and *Coxiella burnetii*) were assayed. 2 samples known to be positive for rheumatoid factor were assayed.
The negative results of the test demonstrated the specific reaction of the kit with no cross reaction or interferences with the referred specimens.

SYMBOLS USED IN LABELS:

IVD	*In vitro* diagnostic medical device
	Use by (expiration date)
	Store at x-yºC
Σ n	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
WELLS X	<X> wells

BIBLIOGRAPHY:

1. Aubert, G., B. Pozzetto, O. G. Gaudin, J. Hafid, A. D. Mbida, and A. Ros. 1992. Evaluation of five commercial tests: complement fixation, microparticle agglutination, indirect immunofluorescence, enzyme-linked immunosorbent assay and latex agglutination, in comparison to immunoblotting for *Mycoplasma pneumoniae* serology. Ann Biol Clin (Paris) 50:593-7.
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REVISED: 06/2014

TICK-BORNE ENCEPHALITIS VIRCLIA® IgG MONOTEST

REF VCM047 Σ 24

CE For *in vitro* diagnostic use

INTENDED PURPOSE

Indirect chemiluminescent immunoassay (CLIA) to test IgG antibodies against TBEV in human serum/plasma.
The test is a qualitative and automated assay, intended to be used as an aid to diagnosis.

INTRODUCTION

Tick-borne encephalitis (TBE) is a human viral infectious disease involving the central nervous system. TBE is caused by the tick-borne encephalitis virus, a member of the family *Flaviviridae*, which includes three subtypes: i) European subtype, endemic in rural and forested areas of central, eastern and northern Europe; ii) Far eastern subtype, endemic in far-eastern Russia and in forested regions of China and Japan; and iii) Siberian subtype, endemic in Urals region, Siberia and far-eastern Russia, and also in some areas in north-eastern Europe. Ticks of the family *Ixodidae* act as both the vector and reservoir for TBEV. The main hosts are small rodents. Humans may also acquire infection by consumption of infected unpasteurised dairy products. The incubation period of TBE is usually between 7 and 14 days. Approximately two-thirds of human TBEV infections are non-symptomatic. In clinical cases, TBE often has a biphasic course. In the viremic phase, symptoms are nonspecific and may include fever, malaise, anorexia, muscle aches, headache, nausea, and/or vomiting. After about 8 days of remission, a second phase of disease occurs in 20% to 30% of patients, when the central nervous system is involved (meningitis, meningoencephalitis, myelitis, paralysis, radiculitis. The diagnosis of TBE is based on the detection of specific IgM antibodies in cerebrospinal fluid and/or serum. TBE antibodies appear 0–6 days after onset and are usually detected when neurological symptoms are present. Specific IgM antibodies can persist for up to 10 months in vaccinees or individuals who acquired the infection naturally. Virus can be detected in blood during the first phase of the disease. As a member of the genus *Flavivirus*, the interference of cross-reactive (but non-neutralizing) antibodies has to be considered.
Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (ChemiLuminescent ImmunoAssay) tests with shorter incubation times while keeping or improving their sensitivity.

TEST PRINCIPLE

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES

All reagents supplied are ready to use.
Serum dilution solution and conjugate are coloured to help in the performance of the technique.
Sample predilution is not necessary.
Reagents required for the run of the test are included in the monodose presentation.

MATERIALS PROVIDED

[1] VIRCLIA® TICK BORNE ENCEPHALITIS IgG MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:
Wells A, B, C: Reaction wells; wells coated with TBEV antigen. Contains inactivated antigen. Contains material of animal origin.
Well D: Conjugate: orange; containing anti-human IgG peroxidase conjugate dilution and 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane as preservatives. Contains material of animal origin.
Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers and 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane as preservatives. Contains material of animal origin.
Well F: Calibrator: clear; positive serum dilution containing 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane as preservative. Contains material of human origin. Contains material of animal origin.
Well G: Substrate component B: clear; containing peroxide.
Well H: Substrate component A: clear; containing luminol.

Special materials required but not provided:
-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR).
-A CLIA automated processor.

STORAGE AND HANDLING CONDITIONS

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY

VIRCLIA® MONODOSE: Once opened, use it in the same day.
Substrate component A is light sensitive. Avoid light exposure. Substrate solutions should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.
VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
6. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. If the kit or its components are stored in the refrigerator, please bring them at room temperature before use.
11. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
12. Keep containers for samples and reagents closed while they are not being handled.
13. Avoid using samples subjected to repeated freeze-thaw cycles.
14. Handle in aseptic conditions to avoid microbial contaminations.
15. Reagents in this kit could include substances of animal and/or human origin and/or inactivated antigen (refer to "Materials provided"). Although materials of human origin have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, all material and patient specimens should be handled and dispose as potentially infectious using safety laboratory procedures. No present method can offer complete assurance that these or other infectious agents are absent. Dispose of unused reagents and waste in accordance with all applicable regulations.
16. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the VIRCLIA® AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
17. Do not use this product in automated processors unless they have been previously validated for that purpose.
18. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Safety precautions.
Observe the following safety information. For further information a Material Safety Data Sheet is available.

Materials provided	Hazardous ingredients:	Hazard statements (CLP):
[1] VIRCLIA® TICK BORNE ENCEPHALITIS IgG MONODOSE	2-Methyl-2H-isothiazol-3-one CAS-No.: 2682-20-4 EC-No.: 220-239-6	H317 – May cause an allergic skin reaction.

Hazard statements (CLP):	H317 – May cause an allergic skin reaction.
Hazard pictograms (CLP):	 GHS07 Health hazard/ Hazardous to the ozone layer
Signal word (CLP):	Warning
Precautionary statements (CLP):	P261 – Avoid breathing dust/fume/ gas/mist/vapours/spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P302+P352 – If on skin: Wash with plenty of water. P321 – Specific treatment (see supplemental first aid instruction on this label). P333+P313 – If skin irritation or rash occurs: Get medical advice/ attention.

CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum/plasma samples are to be refrigerated (2-8°C) upon collection or frozen (-25- -15°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated samples. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PREPARATORY TREATMENT OF THE DEVICE

All reagents supplied are ready to use.
Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting. Once diluted, store at 2-8°C.

ASSAY PROCEDURE

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

INTERNAL QUALITY CONTROL

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS

Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit.
The software of the instrument will validate the values obtained for the controls and display them in the results report. Follow the Operator´s Manual of the Automated Processor. Results cannot be validated if the control values deviate from the expected values.

CALCULATIONS AND INTERPRETATION OF RESULTS

Antibody index= (sample RLU/calibrator RLU)

Index	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 0.9 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 1.1 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS OF USE

1. This kit is intended to be used with human serum/plasma.
2. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by direct diagnostic techniques.
3. This test will not indicate the site of infection. It is not intended to replace isolation.
4. Samples collected at the beginning of infection may not have detectable levels of antibodies. In these cases it is recommended to obtain a second sample between 14 and 21 days to be tested in parallel with the original sample, in order to determine a seroconversion.
5. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
6. A negative result in immunosuppressed patients does not always exclude the possibility of infection.
7. Lack of a detectable antibody level does not exclude the possibility of infection.
8. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
9. The performance of this test has not been evaluated for use in patients without clinical signs and symptoms of infection.
10. Antibody cross-reaction with other flaviviruses is possible.
11. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely in low prevalence scenarios.
12. The performance results showed correspond to comparative studies with commercial predicate devices in a defined population sample. Small differences can be found with different populations or different predicate devices.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

Serum/plasma samples were assayed against a commercial ELISA kit.
The results were as follows:

Samples No.		183
Sensitivity (%)		85
	95% CI	77-90
Specificity (%)		100
	95% CI	94-100
PPV (%)		100
NPV (%)		79
LR+/LR-		-0.86/-0.84

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

WITHIN-RUN PRECISION

3 samples were individually run 10 times each one in a single automated assay in essentially unchanged conditions.
The results were as follows:

Sample	% CV
Positive sample	7.7
Calibrator	7.3
Negative control	6.2

CV: Coefficient of variation

BETWEEN-RUN PRECISION

3 samples were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Sample	% CV
Positive sample	8.4
Calibrator	8.6
Negative control	17.3

CV: Coefficient of variation

INTERFERENCES

Interferences – ANA/RF

10 samples known to be positive for rheumatoid factor and antinuclear antibodies were assayed. No interferences with antinuclear antibodies (5 samples tested) were found. No interferences with rheumatoid factor (5 samples tested) were found.

Interferences – Endogenous substances

3 samples were tested with each interferent. Specifications were fulfilled in all cases. No interferences were found with haemolytic (8.5 g/L hemoglobin), icteric (6 g/L bilirubin) and hyperlipemic (4 g/L cholesterol and 2 g/L tributyrin) samples.

Interferences – Anticoagulants

3 samples were tested with each anticoagulant. Specifications were fulfilled in all cases. No interferences were found with heparin (30 IU/mL), citrate (0.13 mol/L) and EDTA (2 mg/mL).

CROSS REACTIVITY

65 samples known to be positive for other microorganisms (dengue virus, chikungunya virus, Zika virus, cytomegalovirus, Epstein-Barr VCA virus, measles virus, mumps virus, *Borrelia burgdorferi* and *Rickettsia conorii*) were assayed.
No cross-reactivity was found with Zika virus (5 samples tested), cytomegalovirus (10 samples tested), Epstein-Barr VCA virus (10 samples tested), measles virus (8 samples tested), mumps virus (10 samples tested), *Borrelia burgdorferi* (4 samples tested) and *Rickettsia conorii* (6 samples tested). Cross-reactivity was found with dengue virus (3 out of 7 samples tested) and chikungunya virus (3 out of 5 samples tested).

SYMBOLS USED IN LABELS

IVD	*In vitro* diagnostic medical device
	Use-by (expiry date)
y°C / x°C	Store at x-y°C
Σ n	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
	Manufacturer

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TICK-BORNE ENCEPHALITIS VIRCLIA® IgM MONOTEST

REF VCM048 Σ 24

CE For *in vitro* diagnostic use

INTENDED PURPOSE

Indirect chemiluminescent immunoassay (CLIA) to test IgM antibodies against TBEV in human serum/plasma.
The test is a qualitative and automated assay, intended to be used as an aid to diagnosis.

INTRODUCTION

Tick-borne encephalitis (TBE) is a human viral infectious disease involving the central nervous system. TBE is caused by the tick-borne encephalitis virus, a member of the family *Flaviviridae*, which includes three subtypes: i) European subtype, endemic in rural and forested areas of central, eastern and northern Europe; ii) Far eastern subtype, endemic in far-eastern Russia and in forested regions of China and Japan; and iii) Siberian subtype, endemic in Urals region, Siberia and far-eastern Russia, and also in some areas in north-eastern Europe. Ticks of the family *Ixodidae* act as both the vector and reservoir for TBEV. The main hosts are small rodents. Humans may also acquire infection by consumption of infected unpasteurised dairy products. The incubation period of TBE is usually between 7 and 14 days. Approximately two-thirds of human TBEV infections are non-symptomatic. In clinical cases, TBE often has a biphasic course. In the viremic phase, symptoms are nonspecific and may include fever, malaise, anorexia, muscle aches, headache, nausea, and/or vomiting. After about 8 days of remission, a second phase of disease occurs in 20% to 30% of patients, when the central nervous system is involved (meningitis, meningoencephalitis, myelitis, paralysis, radiculitis. The diagnosis of TBE is based on the detection of specific IgM antibodies in cerebrospinal fluid and/or serum. TBE antibodies appear 0–6 days after onset and are usually detected when neurological symptoms are present. Specific IgM antibodies can persist for up to 10 months in vaccinees or individuals who acquired the infection naturally. Virus can be detected in blood during the first phase of the disease. As a member of the genus *Flavivirus*, the interference of cross-reactive (but non-neutralizing) antibodies has to be considered.
Detection methods based on chemiluminescence have received much attention due to their low background, linearity and wide dynamic range. When coupled to enzyme immunoassays, the signal amplification effect provided by the enzyme enables the design of CLIA (ChemiLuminescent ImmunoAssay) tests with shorter incubation times while keeping or improving their sensitivity.

TEST PRINCIPLE

The CLIA method is based upon the reaction of antibodies in the sample tested with the antigen adsorbed on the polystyrene surface. Unbound immunoglobulins are washed off. An enzyme-labelled anti-human globulin binds the antigen-antibody complex in a second step. After a new washing step, bound conjugate is developed with the aid of a chemiluminescent substrate solution that will generate a glow-type luminescence that can be read with a luminometer.

KIT FEATURES

All reagents supplied are ready to use.
Serum dilution solution and conjugate are coloured to help in the performance of the technique.
Sample predilution is not necessary.
Reagents required for the run of the test are included in the monodose presentation.

MATERIALS PROVIDED

[1] VIRCLIA® TICK BORNE ENCEPHALITIS IgM MONODOSE: 24 monodoses consisting of 3 reaction wells and 5 reagent wells with the following composition:
Wells A, B, C: Reaction wells; wells coated with TBEV antigen. Contains inactivated antigen. Contains material of animal origin.
Well D: Conjugate: orange; containing anti-human IgM peroxidase conjugate dilution and 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane as preservatives. Contains material of animal origin.
Well E: Serum dilution solution: blue; phosphate buffer containing protein stabilizers, anti-human IgG and 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane as preservatives. Contains material of animal origin.
Well F: Calibrator: clear; positive serum dilution containing 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane as preservative. Contains material of human origin. Contains material of animal origin.
Well G: Substrate component B: clear; containing peroxide.
Well H: Substrate component A: clear; containing luminol.

Special materials required but not provided:
-VIRCLIA® AUXILIARY REAGENTS (REF:VCMAR).
-A CLIA automated processor.

STORAGE AND HANDLING CONDITIONS

Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY

VIRCLIA® MONODOSE: Once opened, use it in the same day.
Substrate component A is light sensitive. Avoid light exposure. Substrate solutions should not get in contact with acid, combustible materials and strong oxidizing or reducing agents. Make sure that no metal components come in contact with the substrate without having previously tested their compatibility.
VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS

1. For *in vitro* diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
6. Clean pipette tips must be used for every assay step. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. If the kit or its components are stored in the refrigerator, please bring them at room temperature before use.
11. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
12. Keep containers for samples and reagents closed while they are not being handled.
13. Avoid using samples subjected to repeated freeze-thaw cycles.
14. Handle in aseptic conditions to avoid microbial contaminations.
15. Reagents in this kit could include substances of animal and/or human origin and/or inactivated antigen (refer to "Materials provided"). Although materials of human origin have been tested and found negative for Hepatitis B Surface Antigen (HBsAg), Hepatitis C antibodies and Human Immunodeficiency Virus antibodies, all material and patient specimens should be handled and dispose as potentially infectious using safety laboratory procedures. No present method can offer complete assurance that these or other infectious agents are absent. Dispose of unused reagents and waste in accordance with all applicable regulations.
16. Use kit components only. Do not mix components from different kits or manufacturers. Only components of the VIRCLIA® AUXILIARY REAGENTS kit are compatible with all VIRCLIA® references and lots.
17. Do not use this product in automated processors unless they have been previously validated for that purpose.
18. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Safety precautions.
Observe the following safety information. For further information a Material Safety Data Sheet is available.

Materials provided	Hazardous ingredients:	Hazard statements (CLP):
[1] VIRCLIA® TICK BORNE ENCEPHALITIS IgM MONODOSE	2-Methyl-2H-isothiazol-3-one CAS-No.: 2682-20-4 EC-No.: 220-239-6	H317 – May cause an allergic skin reaction.

Hazard statements (CLP):	H317 – May cause an allergic skin reaction.
Hazard pictograms (CLP):	 GHS07 Health hazard/ Hazardous to the ozone layer
Signal word (CLP):	Warning
Precautionary statements (CLP):	P261 – Avoid breathing dust/fume/ gas/mist/vapours/spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P302+P352 – If on skin: Wash with plenty of water. P321 – Specific treatment (see supplemental first aid instruction on this label). P333+P313 – If skin irritation or rash occurs: Get medical advice/ attention.

CONDITIONS FOR COLLECTION, HANDLING AND PREPARATION OF THE SPECIMEN

Blood should be collected aseptically using venipuncture techniques by qualified personnel. Use of sterile or aseptic techniques will preserve the integrity of the specimen. Serum/plasma samples are to be refrigerated (2-8°C) upon collection or frozen (-25- -15°C) if the test cannot be performed within 7 days. Samples should not be repeatedly frozen and thawed. Do not use hyperlipemic, hemolysed or contaminated samples. Samples containing particles should be clarified by centrifugation. The kit is suitable for use with serum or plasma.

PREPARATORY TREATMENT OF THE DEVICE

All reagents supplied are ready to use.
Only the VIRCLIA® WASHING SOLUTION included in the auxiliary component kit VIRCLIA® AUXILIARY REAGENTS must be prepared in advance. Fill 50 ml of VIRCLIA® WASHING SOLUTION (20x) up to 1 litre with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting. Once diluted, store at 2-8°C.

ASSAY PROCEDURE

1. Bring VIRCLIA® WASHING SOLUTION (diluted according to the instructions) to room temperature before use (approximately 1 hour).
2. Follow the Operator´s Manual of the Automated Processor.

INTERNAL QUALITY CONTROL

Each batch is subjected to internal quality control (Q.C.) testing before batch release complying with specifications stricter than validation protocol for users. Final Q.C. results for each particular lot are available.
The control material is traceable to reference sera panels internally validated.

VALIDATION PROTOCOL FOR USERS

Each monodose includes one calibrator (well A) and one dilution of the calibrator used as negative control (well C). It allows the validation of the assay and kit.
The software of the instrument will validate the values obtained for the controls and display them in the results report. Follow the Operator´s Manual of the Automated Processor. Results cannot be validated if the control values deviate from the expected values.

CALCULATIONS AND INTERPRETATION OF RESULTS

Antibody index= (sample RLU/calibrator RLU)

Index	Interpretation
<0.9	Negative
0.9-1.1	Equivocal
>1.1	Positive

Samples with equivocal results must be retested and/or a new sample obtained for confirmation.
Samples with indexes below 0.9 are considered as not having antibodies of the specificity and class measured by this kit.
Samples with indexes above 1.1 are considered as having antibodies of the specificity and class measured by this kit.

LIMITATIONS OF USE

1. This kit is intended to be used with human serum/plasma.
2. The results of samples should be used in conjunction with clinical evaluation and other diagnostic procedures. A definitive diagnosis should be made by direct diagnostic techniques.
3. This test will not indicate the site of infection. It is not intended to replace isolation.
4. Samples collected at the beginning of infection may not have detectable levels of antibodies. In these cases it is recommended to obtain a second sample between 14 and 21 days to be tested in parallel with the original sample, in order to determine a seroconversion.
5. Results in IgG detection in neonates must be interpreted with caution, since maternal IgG is transferred passively from the mother to the foetus before birth. IgM assays are generally more useful indicators of infection in children below 6 months of age.
6. A negative result in immunosuppressed patients does not always exclude the possibility of infection.
7. Lack of a detectable antibody level does not exclude the possibility of infection.
8. Reliable results are dependent on adequate specimen collection, transport, storage and processing procedures.
9. The performance of this test has not been evaluated for use in patients without clinical signs and symptoms of infection.
10. Low levels of IgM antibodies may occasionally persist for more than 12 months post-infection.
11. Antibody cross-reaction with other flaviviruses is possible.
12. Positive and negative predictive values are highly dependent on prevalence. False negative test results are more likely when prevalence of disease is high. False positive test results are more likely in low prevalence scenarios.
13. The performance results showed correspond to comparative studies with commercial predicate devices in a defined population sample. Small differences can be found with different populations or different predicate devices.

PERFORMANCE CHARACTERISTICS

SENSITIVITY AND SPECIFICITY

Serum/plasma samples were assayed against a commercial ELISA kit.
The results were as follows:

Samples No.		170
Sensitivity (%)		98
	95% CI	91-100
Specificity (%)		99
	95% CI	95-100
PPV (%)		98
NPV (%)		99
LR+/LR-		-1.00/-0.98

CI: Confidence intervals
PPV: Positive predictive value
NPV: Negative predictive value
LR+: Positive likelihood ratio
LR-: Negative likelihood ratio

WITHIN-RUN PRECISION

3 samples were individually run 10 times each one in a single automated assay in essentially unchanged conditions.
The results were as follows:

Sample	% CV
Positive sample	7.5
Calibrator	6.3
Negative control	6.0

CV: Coefficient of variation

BETWEEN-RUN PRECISION

3 samples were individually run on 5 consecutive days in 2 different automatic processors.
The results were as follows:

Sample	% CV
Positive sample	9.9
Calibrator	6.3
Negative control	25.6

CV: Coefficient of variation

INTERFERENCES

Interferences – ANA/RF

10 samples known to be positive for rheumatoid factor and antinuclear antibodies were assayed. No interferences with antinuclear antibodies (5 samples tested) were found. No interferences with rheumatoid factor (5 samples tested) were found.

Interferences – Endogenous substances

3 samples were tested with each interferent. Specifications were fulfilled in all cases. No interferences were found with haemolytic (8.5 g/L hemoglobin), icteric (6 g/L bilirubin) and hyperlipemic (4 g/L cholesterol and 2 g/L tributyrin) samples.

CROSS REACTIVITY

54 samples known to be positive for other microorganisms (dengue virus, chikungunya virus, Zika virus, cytomegalovirus, Epstein-Barr VCA virus, measles virus, mumps virus, *Borrelia burgdorferi* and *Rickettsia conorii*) were assayed.
No cross-reactivity with chikungunya virus (5 samples tested), Zika virus (5 samples tested), cytomegalovirus (3 samples tested), Epstein-Barr VCA virus (9 samples tested), measles virus (5 samples tested), mumps virus (8 samples tested), *Borrelia burgdorferi* (5 samples tested) and *Rickettsia conorii* (6 samples tested). Cross-reactivity with dengue virus (2 out of 8 samples tested) was found.

SYMBOLS USED IN LABELS

IVD	*In vitro* diagnostic medical device
	Use-by (expiry date)
y°C / x°C	Store at x-y°C
Σ n	Contains sufficient for <n> test
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
	Manufacturer

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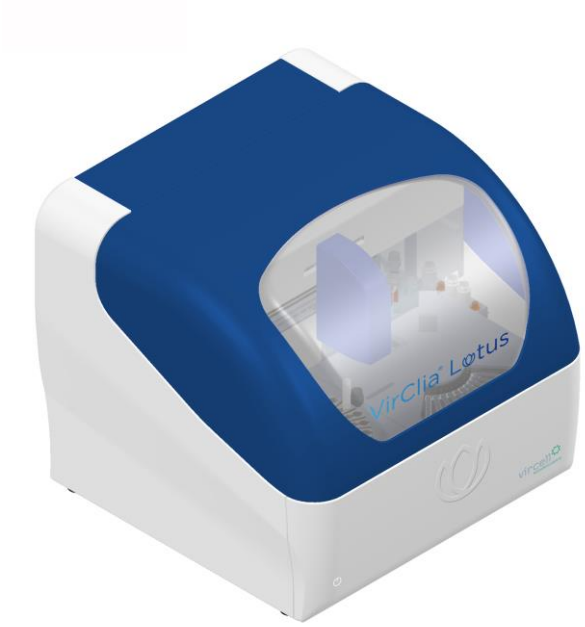
User Manual

VirClia® Lotus



IVD

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VirClia® Lotus MODELS

This manual applies to the following VirClia Lotus™ models:

Order Code	Model Description
VCLTS-CL	VIRCLIA LOTUS

TECHNICAL ASSISTANCE

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Standard applied in this document:
UNE EN ISO 18113-3:2012
IEC 61010-1 II Edition File 6290 (October, 2013)
IEC 61010-2-101 I Edition File 7134 (November, 2003)

IMPORTANT

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The information about the VirClia Lotus ™ analyzer included in this user manual is built on the knowledge and accumulated expertise of Vircell, S.L. It is the user who bears the full responsibility for the good or bad use of the VirClia Lotus ™ analyzer instrument. This manual contains all the information needed to perform the routine operations of the instrument. Therefore, the user must read carefully this user manual before using the instrument. Besides this, please read the reagents package inserts to be used during the process. Vircell, S.L. or any other affiliate companies are in no case liable for any injuries, loss or damages caused by bad practices, intentional acts and omissions or gross negligence of the user.

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The software used in the VirClia Lotus ™ equipment has been designed so that any personal data introduced is stored dissociated or pseudonymized **by the user** according to the definitions provided by the Regulation EU 2016/679 (GDPR). It is the user´s **sole** responsibility to maintain and treat the data according to the applicable legislation of data protection in the reference territory or the Regulation EU 2016/679 (GDPR) in Europe. Vircell, S.L. declines any responsibility which could result from the violation of this liability exemption clause for any infringement of the aforementioned regulations. For any query that may arise in connection with the personal data protection policy, please contact personaldata@vircell.com or customerservice@vircell.com.

In order to protect the integrity of the results stored in VirClia® Lotus, the software incorporates cybersecurity measures which allow the notification of access by unauthorized personnel and makes it difficult to modify and manipulate the recorded raw data.

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USE OF THIS MANUAL SHALL BE CONSIDERED ACCEPTANCE OF THE TERMS AND CONDITIONS SET FORTH HEREIN.

Chapter 1

1. INTRODUCTION

1.1 PREFACE

This document and all annexes thereto shall form an integral part of the VIRCLIA LOTUS ™ instrument. Do not use the instrument without having read and understood all the information provided in this user manual.

The manufacturer recommends the user to read every section of the User Manual. Special attention must be paid to the **NOTES** used to describe important information for a proper use of VirClia Lotus™ instrument and **WARNINGS** used to emphasize potential risks or dangers deriving from the instrument use.

The manufacturer disclaims any responsibility for any partial or total unauthorized copies of the present Manual.

1.2 SYMBOLS

Legend of graphic symbols used on the Instrument

IVD	*In vitro* diagnostic medical device.
MET c us	Instrument satisfying MET standards for the U.S.A. and Canadian market
	Date of manufacture of the unit.
SN	Serial number of the unit.
	Manufacturer data.
(01) 08436040328286 (11) YYMMDD (21) XXXX	UDI label. (01): Instrument ID. (11): Date of manufacture. (21): Instrument serial number.

Legend of Electrical and Safety symbols used on the instrument

	Protective earth; protective ground.
	Recycle: Electronic Equipment – Do not throw in trash, WEEE: Waste Electrical and Electronic Equipment (Directives 2002/96/CE and 2003/108/CE)
	Caution: read the Manual carefully and pay special attention to the safety symbols.
	Warning; Electricity: risk of electric shock.
	Warning; hot surface.
	Warning; biological hazard.
	Warning; cut hazard.
	Warning; crushing of hands.
	Warning; laser beam hazard

1.3 INTENDED USE

The VIRCLIA LOTUS ™ instrument, with its "**Lotus**" application software integrated on it, is an analyzer designed for the automation of medical diagnostic techniques performed with *in vitro* chemiluminescence immunoassay (CLIA), on special VirClia® Monotest Devices (VirClia Strips).

Figure 1.3-01. VirClia® Monotest Device Sample

VirClia Lotus ™ automates the steps of the diagnostic tests in a rapid, accurate and precise sequence. It includes the dilution and dispensing of samples and controls, dispensing of reagents, incubation, shaking, washing, reading, processing, interpretation and archiving of results.

Read all instructions, terms and conditions detailed in this User Manual, prior to operating the instrument. The customer shall follow the manufacturer instructions for performing the diagnostic test in order to minimize the chance of errors. If the user modifies the instructions, the test shall no longer meet the requirements for VirClia Lotus ™ appropriate results.

VirClia Lotus ™ analyzer is intended only for professional *In Vitro* Diagnostic use and the system shall work properly only with VirClia® chemiluminescence assays. Therefore, tests shall not be performed with VirClia Lotus ™ if reagents are not provided or authorized by Vircell, S.L.

Users are not allowed to make any change neither to the instrument nor to the software without authorization from Vircell, S.L. In no event shall Vircell, S.L. be liable for any direct, consequential, incidental or indirect damage related to the use of different reagents to VirClia® or any change made in the instrument without the aforementioned authorization. meaning a lack of conformity to the applicable regulations declared by the manufacturer. In this case, the customer will be exclusively responsible for the fulfilment of the applicable regulations as well as all the possible legal liabilities derived from any infringement of the laws in force. All intellectual property rights are and shall remain Vircell, S.L. exclusive property.

1.4 GENERAL DESCRIPTION

VirClia Lotus ™ uses closed system programmed to process diagnostics immunoassays based on VirClia® Monotest Devices (VirClia Strips). For each assay, there is a specific Monotest Device, and method. For each sample, one or more assays can be processed in order to determine one or more parameters in the same analysis session. It means that in order to process one sample, one or more VirClia® Monotest Devices shall be loaded on the instrument. The VirClia Lotus™ is able to support **continuous loading** of the samples and Monotest Devices: It shall allow the user to add new samples and devices and to program their assays even while the instrument is processing those previously loaded, due to its high flexibility.

Figure.1.4-01 'Front View (Door Closed)'

Figure 1.4-02 'Front View (Lids Open)'

Legend:

①	Sample Rotor	⑥	Wash Buffer Tank
②	Sample Needle Arm	⑦	Distilled Water Bottle
③	Strip Rotor	⑧	Auxiliary Reagent Rack
④	Sample Needle Washing Cup	⑨	Cover Locks
⑤	Strip Waste Container	⑩	Waste Tank

1- **Sample Rotor:** Rotor where the samples tubes, pre-dilution cuvettes and diluent bottles used during the assay are placed**.**

2- **Sample Needle Arm for sample preparation:** This arm holds the needle used in the first pipetting stage.

3- **Strip Rotor:** The strips needed during the assay shall be located in this rotor.

4- **Sample Needle Washing Cup:** This is the washing station for the sample needle where the first pipetting stage shall be carried out. Both the inner and outer side of the needle are cleaned in this washing station.

5- **Strip Waste Container:** A waste container where the used strips are discarded automatically after the assay.

6- **Wash Buffer Tank:** The washing solution prepared according to manufacturer specifications is contained in this tank with a capacity up to 5 liters. The instrument contains a scale to weigh the wash buffer solution.

7- **Distilled Water Bottle:** The Distilled water used during the maintenance is stored in this tank. The capacity is 0.25 liters. The instrument contains a scale to weigh the Distilled water.

8- **Auxiliary Reagent Rack:** Racks used for additional reagents such as avidity and/or decontamination solution.

9- **Cover Locks**: Electromagnets used for the instrument cover locking during the assay.

10- **Waste Tank**: This tank is used for the liquid waste generated during the assay. The capacity is 5 liters. The instrument contains a scale to weigh liquid waste.

The VirClia Lotus ™ is equipped with three rotors, being two of them located in the user accessible area. The first rotor is used to load the Samples Tubes and Pre-dilution Cuvettes together with the Diluent Bottles (*Sample Rotor*); the second one is used to load the Monotest Devices (*Strip Rotor*). The third rotor is called the *Reaction Rotor* and is where the assay procedure is actually performed. The Monotest Devices Washing Unit, the Reagent Dispensing Needle, the Reagent Needle washing cup, the Shakers and the Reader are located over it. Moreover, the Reaction Rotor warms up in order to perform the incubation of the Monotest Devices.

1.5 TECHNICAL SPECIFICATIONS

POWER SUPPLY	US/CAN AC: 110 - 120 V ~ 60HZ AC: 110 - 230 V ~ 50-60HZ		
ELECTRICAL POWER CONSUMPTION	365 VA		
FUSES	2 X 10.0 A T (5 X 20 MM)		
DIMENSIONS	750x740x650mm (LENGTH/ WIDTH / HEIGHT)		
WEIGHT	70 Kg/155 lbs (MAX)		
AMBIENT TEMPERATURE	OPERATIONAL	FROM +18°C TO + 35°C	OPTIMAL: 20-25ºC
	STORAGE	FROM + 5°C TO + 45°C	
RELATIVE HUMIDITY THRESHOLD	FROM 20 TO 80% WITHOUT CONDENSATION		

Altitude	from 0 to 3000 meters
Noise Level	< 75db (Decibel)
Heat output	500 BTU/Hour
Embedded Cpu Board	STM32F4 processor with 8MB SD-RAM, 1MB FLASH, 1KB EEPROM, micro-SD card for internal file storage
Peripheral Control Units	Microprocessor board on host Bus
Interface	1 USB Client for host communication
Samples – Capacity	50 sample tubes
Monotest Devices Strips – Capacity	39 in the strip loading Rotor + 40 in the Reaction Rotor
Diluent Bottles - Capacity	6 bottles
Pre-dilution Cuvettes - Capacity	50 cuvettes
Reagent bottles – Capacity	5 + 1 bottle for decontaminant solution for samples needle
Wash tank - Capacity	5 Liters x 1 Tank
Distilled water – capacity	0.25 Liters x 1 bottle
Waste tank - Capacity	5 Liters x 1 Tank
Strip waste container – Capacity	60 strips
External tank	11 Litres
Protection Category	Class I
Safety Standards	IEC 61010-1 (Ed.2013-10); CAN/CSA-C22.2 Nr.61010-1-04 (Ed.2004-07); UL61010-1 (Ed.2004-07)
EMC Standards	IEC 61326-1 (Ed.2013-07); IEC 61326-2-6 (Ed.2014-06)
Installation category	II

The safety and performance of this instrument shall not be ensured if a power supply cable other than the one supplied is used. (Even if compatible with power supply network of the installation country).

The analyzer contains nr. 1 battery Li-Mh CR2032 on the CPU board.

WORKSTATION MINIMUM REQUIREMENTS:

COMPUTER	• INTEL CORE I3 /2.8 GHZ PROCESSOR PERSONAL COMPUTER • 4GBYTE RAM • 256 GB HARD DISK • 100/1000 MBIT ETHERNET RJ45 PORT • FULL HD CAPABLE DISPLAY OUTPUT PORT • 4 USB PORT •AUDIO-OUT CONNECTOR
KEYBOARD	USB ALPHANUMERIC (STANDARD)
MOUSE	USB MOUSE
VIDEO MONITOR	(1920 X 1080) FULL HD, (PREFERABLY TOUCH SCREEN MONITOR)
SPEAKERS	2 CHANNELS
PRINTER	ANY PRINTER SUPPORTED BY THE OPERATING SYSTEM
OPERATING SYSTEM	WINDOWS 10 PROFESSIONAL X64

UPS FEATURES (FOR **ONE HOUR** OF AUTONOMY):

DIMENSIONED TO SUPPLY ANALYZER, COMPUTER, PRINTER AND MONITOR

RECOMMENDED NOMINAL POWER	1100 VA
WORKING MODE	ON-LINE WITH DOUBLE CONVERSION, NO INTERVENTION DELAY.
MINIMUM BATTERY CAPACITY	14 Ah
OUTPUT CHARACTERISTICS: • VOLTAGE DISTORTION WITH NON-LINEAR LOAD • WAVEFORM • CURRENT CREST FACTOR	 < 4% SINUSOIDAL 3 : 1
INTERFACE	SERIAL OR USB PORT. A SUPERVISION SOFTWARE MUST BE SUPPLIED FOR MICROSOFT WINDOWS OPERATING SYSTEMS: WINDOWS 10

To identify the right UPS for the instrument installation country, refer to the local country features network power supply.

Chapter 2

2. INSTALLATION

2.1 TECHNICAL ASSISTANCE TASKS / MATERIALS REQUIRED

The following steps must be taken prior to any service related to the instrument or maintenance.

a) TURN THE POWER SWITCH TO "OFF", LOCATED ON THE RIGHT SIDE OF THE INSTRUMENT

b) UNPLUG THE INSTRUMENT FROM THE UPS POWER SUPPLY OR POWER OUTLET TO GARANTY YOUR SAFETY FROM ELECTRICAL AND MECHANICAL HAZARDS

FAILURE TO FOLLOW THE ADVICE AND INSTRUCTIONS IN THIS DOCUMENT RELEASES THE MANUFACTURER FROM ALL LIABILITY AND MAY CAUSE DAMAGE TO THE INSTRUMENT.

MATERIALS REQUIRED / INSTRUMENTATION:

TOOLS REQUIRED FOR MAINTENANCE:

Digital multimeter
HARTA STRIP (Recommended)
Temperature sensor
Dye kit
EVVALS kit (for preventive maintenances, VTK kit is accepted instead)
Phillips screwdrivers set
Allen wrenches set
5.5 mm and 7 mm Socket wrenches
Set of open-ended spanners or wrenches (including 5.5mm and 5mm)
Circlips pliers
Tweezers
RS232 to USB Converter

[CODE 3050] ASSISTANCE KIT COMPOSED BY:

[CODE 11400060] LOCTITE 243 Thread Locker (Medium-strength).
[CODE 12400040] Lubricant Spray WD-40 200ml.
[CODE 19900860] Special Allen wrench 2.5-3 mm T-Handle.

2.2 PRELIMINARY CHECK

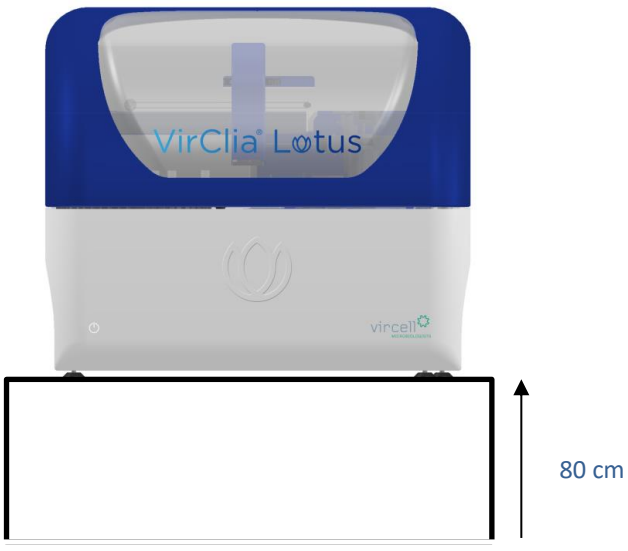
The following conditions are required to certify the operator and instruments safety:

- The power supply network (Category II Installation), must be "compatible" with the specifications of voltage and current specifications shown on the identification label affixed on the side of the instrument. To ensure the proper functioning of the instrument, please check the electrical output system of the building before installation.
- The electrical system outlet must be properly grounded as defined by the standards for outlet electrical installation.
- Before making any connection, verify compatibility with external equipment, such as the host or PC (with the user manual) and remember to connect the cables only with external devices with the switch in the off position. Verify continuity and grounding of the electrical system.
- IT IS COMPLETELY FORBIDDEN to remove or modify or override any of the safety devices installed on the instrument.

It is advisable to connect the analyzer to an Uninterruptible Power Supply (UPS) to ensure the normal operation in case of an electrical system failure.

2.3 PLACEMENT

The VirClia Lotus™ analyzer is intended for use in clinical and research laboratories.
The instrument must be located away from any source of heat, and must be in a dust-free environment, on a level surface, free from vibrations and oscillations on the workbench.
It is advisable to use a workbench with a sufficient range of weight tolerance and a height of 80 cm to ensure an ergonomically correct position for the user during the loading and extracting operations in the instrument and adjacent working area.



The VirClia Lotus™ is compliant with the electromagnetic compatibility directives; however, it is advised that the instrument should be placed on an individualized circuit and not on circuits with other possible electromagnetic sources such as refrigerators, laboratory centrifuges, or instruments without CE marking that may interfere with the correct operation of the instrument.

The instrument requires a placement of at least 20 cm away from the wall, to have access to the main cables and tubing on the external side of the instrument and thus be able to access the instrument in order to intervene quickly in case of instrument malfunction. The instrument cover must remain unobstructed and accessible. **Do not store any material on top of the instrument**.

If you do not use the instrument for an extended period of time, it is advised to perform a decontamination/cleaning of the internal parts as described in the user manual and then disconnect the power supply cable and protect the instrument and its peripherals from dust.

2.4 CONNECTION

To connect the main power supply cable:

1. Verify the OFF position of the main switch.
2. Connect the main power supply cable to the main inlet located on the right side of the instrument, and then plug it into the power supply outlet or at the UPS unit.

To connect the PC:

1. Place the Personal Computer, Keyboard, Monitor and Printer near the Instrument.
2. Connect the USB 2.0 compliant Cable* to the USB host port of the PC.
3. Connect the other end of the cable into the USB port of the device, located on the right side of the instrument.
4. Connect the keyboard, monitor and printer to the PC.
5. Connect the PC and monitor to the mains power supply or to the UPS unit.

* The cable for the external connection must not exceed 2 meters in length.

2.5 SOFTWARE – DESIGN AND SPECIFICATIONS

Description and requirements specification

The software used in this equipment operates according to the user interface of the instrument. This software allows creating and executing work lists, analyse the results of different assays, and verify the results obtained.

This software allows connection to laboratory computer system (LIS) using VirCom middleware.

VirClia® Lotus System includes different acoustic sounds and visual signals to warn of different situations when user action is needed.

The software package containing the program necessary for the correct performance of the instrument is installed on the PC, ready for use.

The application has been developed according to the EN 62304:2007 standard. This standard establishes a classification of the software on the basis of its critical nature and potential impact on the patient health / clinical status. The software system is software safety class B.

The instrument includes a CPU board that allows the autonomous completion of a run once it has been launched. This CPU board includes an STM32f4 processor with 8mb SD-RAM, 1mb flash, 1kb EEPROM, micro-SD card for internal file storage with 8GB capacity (HC1) type 10.

Chapter 3

3. OPERATING PROCEDURE

3.1 BEFORE USING THE INSTRUMENT

General Warnings

Operators working with VirClia Lotus™ shall have a good working knowledge of *In Vitro* Diagnostics tests (IVD).

When using VirClia® Lotus, the safety instructions must be followed. It is necessary to read this manual prior to using the instrument. VirClia® Lotus has been designed following safety protocols for electric devices specified in the aforementioned certifications, in order to guarantee the correct performance of the instrument, both electrically and mechanically, under normal conditions of use.

Ensure that the instrument is closed, its internal parts inaccessible and the protective covers and any safety components of the equipment in place, when the trial is underway.

It is important to follow all the instructions indicated in this manual, in order to achieve accurate and reliable results from the assays performed in this instrument.

To ensure the correct use of VirClia Lotus™, it is recommended to follow the general precautions listed below:

- The installation and service must be carried out by qualified and trained Technical Service. Only the authorized Technical Service or the person responsible for the instrument must transport or move the equipment, as it contains fragile material. It is not allowed to override the safety and protection devices of the access door (microswitch and electromagnetic sealing) from VirClia Lotus™.
- Any adjustment, service or repair should be performed by qualified and trained Technical Service. Failure in doing so, may cause warranty invalidation.
- Do not install any spare parts not provided by Vircell S.L.
- Do not attempt to modify the Software or the Operating System. The required Software and Operating System updates are provided by Vircell S.L.
- In case of VirClia Lotus™ malfunction, stop using the instrument and contact immediately the Technical Service.
- Consult this User Manual, before running the tests. Keep the User Manual at hand in the laboratory for easy consultation, if needed.
- This manual may include information on the use of accessories and components approved by the manufacturer. The instrument is designed to be used only with reagents established by the manufacturer and only these reagents should be used to ensure proper operation of the equipment.
- This manual contains information about operational programs and includes errors messages and warnings that may appear during the operating procedure, indicated in section 4 “Errors and Warning messages”.
- Section 3.1 provides information on inspection, cleaning and control to be done prior to use.
- Cleaning should be performed following the instructions indicated in in this user manual. Before connecting the equipment, make sure that it is operated using a power source with an operating voltage compatible with the requirements stated on the device label.
- Avoid direct contact with materials that may be infectious or represent other risks to the human body. Wear appropriate personal protective equipment (PPE) while handling or cleaning the instrument. If biological material is spilled on or inside the instrument, clean it according to sanitary regulations. Consult applicable local regulations prior to

disposal of materials. The bench space recommended to accommodate the instrument is of at least 20 cm (8 inches) at the back of the machine for proper ventilation. Periodically maintenance includes cleaning and disinfection procedures to be performed by the user. Check the maintenance section of this manual to see the list of maintenance operations and their frequency.

- The user must comply with the chemical and biological hazard protection regulations, as specified in the health precautions section of this User Manual, during handling and loading of samples, controls, specific reagents, calibrators and thinners, and also during the maintenance operation, decontamination, disinfection procedures and disposal of parts of the instrument or the instrument as a whole.

- It is essential that all kits instructions are followed when running the instrument, paying special attention to the sample type and interfering substances.

- Do not use the instrument if it does not work correctly. Electrical connections (plugs, sockets and connectors) conduct electric current. Live parts can cause electrical shock, so please take care to observe all applicable safety instructions, stated on section 3.1 – "Electrical Safety". Use a separate power outlet for the equipment. To reduce the risk of electric shock, never handle the inside of the equipment without having disconnected it from the power supply. Always disconnect the equipment from the power supply before proceeding to any kind of maintenance. Optimum performance of VirClia Lotus™ depends on the preventive maintenance being performed by qualified and trained Technical Service. Ensure that the unit is disconnected from the power supply before any kind of maintenance, having allowed the temperature zones to cool.

- Section 5 of this manual describes the importance of proper maintenance. Periodic maintenance procedures, provided to limit the effects of wear and/or aging, are also described in the service manual. The risks associated with the improper maintenance of the instrument have been highlighted in this manual. The dangers derived from malfunction, maintenance and aging can contribute to a decrease in the safety of the user and the patient.

- The person responsible for the instrument must ensure that the ambient light allows the correct lighting of the working areas, both for their configuration, as well as to execute the routines and maintenance. The team leader must ensure that safety distances around the equipment are maintained. Locate the equipment in an optimal position, so that it should not be exposed to water or steam,

- Make sure that the instrument is positioned away from any air conditioning outlets or heat exchangers.

- Avoid exposing the equipment to extreme temperature, humidity or dust.

- Place the instrument on a level, vibration or shock free workbench.

- Refer to Section 2 – "Installation" to check the limits for installation, operation, transport, storage, placement of the instrument, known interferences and the uses not allowed by the manufacturer (Warnings and Precautions). The manufacturer has prepared a Service Manual in which a specific installation protocol has been defined, which provides for a series of calibration and verification tests to ensure that valid and reliable results are obtained for an analysis session. These procedures shall be carried out by qualified and trained Technical Service.

- Refer to Section 3.1 – "Before Using the Instrument", to check the kind of tubes compatible with the instrument. It is important to act with caution, when loading the equipment with the sample tubes.

- Do not touch high temperature areas. Contact with these areas may cause burns.

- Never reuse single-use medical devices.

- This manual also covers aspects important for the installation of the instrument, describing the correct installation procedures, taking into account all the risks associated with an incorrect storage and also providing the handling instructions of the instrument.

- Take care of all local regulations in force regarding electrical and electronic waste. Refer to section 3.1 – Before Using the Instrument, to check that the instrument is duly disposed in environmentally sound manner and ensure the equipment is free of dangerous and harmful substances (decontaminated and disinfected).

Health Hazards

The VirClia Lotus™ use does not exclude the exposure to biohazardous materials.

For safety measures, follow the instructions described below:

- Always wear appropriate personal protective equipment (PPE), including eye protection, gloves, mask and a lab coat when handling human samples, specific reagents, controls, calibrators and diluents, in order to prevent from cuts, abrasions and any other type of skin lesions adequately.
- Do not pipette samples by mouth. Do not eat, drink, smoke or apply cosmetics in areas where samples and/or reagents are handled.
- Corrosive substances may cause burns and eye damage, so avoid exposure to this kind of substances and always be aware of the potential risks of any type of substance. Always use PPE when handling reagents such as VIRCLIA® DE-CONTAMINATION SOLUTION, as it contains 0.5 M sulphuric acid.
- Wear personal protective equipment, eye protection and disposable gloves, when handling hazard substances and always wash hands thoroughly thereafter

Keep in mind that some reagents are potentially hazardous chemicals under applicable regulations. The user must follow the protection regulations for chemical and biological hazards under the following conditions:

- When handling withsamples, controls, specific reagents, calibrators and diluents
- During the maintenance operations;
- In case of spillage or leakage of potentially hazardous materials within the working area of the instrument.
- During the procedures for decontamination, disinfection and disposal of parts of the instrument or the instrument as a whole.
- The user is recommended to read carefully what is specified on the labels of the VirClia kits and in the Reagent Vials and take into account the indications contained in the Safety Data Sheet included in each of the kits.

Electrical Safety

Vircell S.L. declares that all internal parts of VirClia Lotus™ have been designed and implemented to avoid any electric risk to the user.

WARNING: The risk of electrical shock hazard exists if the grounding terminal connection is damaged or disconnected. In this case, do not use the instrument until the grounding terminal is totally restored.

- Before any connection, ensure there is a proper grounding connection.
- Extension sockets and serially connected adapters should not be used.

WARNING: Any repair must be performed by qualified and trained Technical Service. Do not open the electronics board, on the left front part of the instrument, without first disconnecting the power cables.

WARNING: The Manufacturer does not guarantee the safety and performance requirements in case the VirClia Lotus™ is powered by a cable that, although compatible with the voltage of the country where the instrument is installed, it is not the one supplied by the manufacturer.

- In case of malfunction or evident damages, disconnect the power cable and contact the Technical Service of your local distributor.
- Use the instrument only as described in this VirClia Lotus™ Manual. Any use other than the one for what the instrument is intended, should be considered inappropriate and may reduce the protection offered by the instrument.
- The Manufacturer is in no case liable for any damage that may arise from an improper, incorrect and / or unreasonable use of the instrument or if the instrument is used in electrical systems that do not comply with the safety regulations in force in the country where it is installed.

Environmental Requirements

Install VirClia Lotus™ on a level surface in a place where a constant temperature and an adequate humidity level is guaranteed. Avoid direct exposure to sun light. Connect the VirClia Lotus™ plugs to the power outlets with grounded connection.

Ensure that VirClia Lotus™ is only carried out with compatible voltages established by the technical characteristics of the instrument. All these requirements are indicated in the Technical specification Section.

Waste Disposal

The disposable accessories and consumables that may be contaminated during the use of the instrument, must be handled and disposed as biological waste in accordance with the safety laboratory procedures and local regulations. All materials used should be handled and disposed of as potentially infectious materials. Follow local regulations for the disposal of clinical waste.

Sample Preparation

Verify that there are no clots, foam or bubbles in the samples before the test is run.

NOTE: The information on sample preparation provided in this Manual, is of general nature. The operator must carefully read the instructions for use included in each kit.

WARNING: Improper sample preparation may lead to false negatives. Samples that potentially contain particles or clots must be centrifuged or clarified before being used.

Sample Type

VirClia Lotus™ automatically performs *in vitro* diagnostics tests on samples such as blood, serum and other biological fluids.

NOTE: It may be necessary to validate the pipetting of samples with abnormally high viscosities. Consult the Technical Service for more information.

Storage

Samples must be stored following the instructions given by the kit manufacturer.

Sample Identification

VirClia Lotus™ is equipped with a barcode reader on the external side of the Sample Rotor. Barcode samples are identified through the barcode reader. The software and the hardware have been designed to avoid exchange of samples once scanned. Excessively large barcode labels may require to select one of the three available scanning procedures to guarantee a correct sample barcode identification. Consult your local provider in case excessively large barcode labels are used.
The following codes are recognized by the barcode reader of VirClia Lotus™:

Code	Default Status
UPC-A	Enabled
UPC-E	Enabled
EAN-13	Enabled
EAN-8	Enabled
Code 39	Enabled
Code 93	Enabled
Code 128	Enabled
UCC/EAN-128	Enabled
Codabar	Enabled
Interleave 2 of 5	Enabled
Standard 2 of 5	Enabled
Matrix 2 of 5	Enabled
Industrial 2 of 5	Enabled
Chinese Postal	Enabled
MSI/PLESSEY	Enabled

Minimum volume of sample required

The minimum volume of sample is the dead volume plus the sample volume needed to run the assay. The preprogramed tubes in the VirClia Lotus™ software are as follows:

75mm x 11mm	100mm x 11mm
75mm x 12mm	100mm x 12mm
75mm x 13mm	100mm x 13mm
75mm x 16mm	100mm x 16mm
2 mL Sarsted tube (to be used with Vircell Adapter)	

NOTE: Tubes exceeding 16 mm in diameter and/or 100 mm in height cannot be programmed.

Dead volume

If the sample volume is less than or equal to the minimum volume measured by the software, it cannot be safely aspirated or detected by the pipetting system. The dead volume is around 200 µL for standard test-tubes. This value can be reduced by using test-tubes with smaller diameters. In this case, the calibration of the test-tubes must be performed by technical staff authorized and trained by VIRCELL S.L.

WARNING: The use of test-tubes other than those for which VirClia Lotus™ was calibrated may lead to false negative results due to errors in the calculation of samples volumes.

3.2 BEFORE PROGRAMMING AN ANALYSIS SESSION

Before running each session, the following operations must be carried out:

- Turn on the switch located on the right side of the instrument.
- Turn on the computer and make sure all the workstation connections are correct (printer, mouse, keyboard and video).
- Make sure the strip waste container is empty.
- Make sure the waste tank is empty.
- Make sure the drain tube is connected to the specific external tank or to the central drain, if available.

3.3 SESSION PROGRAMMING, RUNNING AND RESULTS

3.3.1 WASH BUFFER, CLEANING SOLUTION, AND WASTE TANK

Before starting the PC program, make sure that the wash buffer solution has been prepared following the dilution ratio specified in the Instructions for Use.

3.3.1.1 FILL THE WASH BUFFER TANK (5L CAPACITY)

Once the tank is filled with Wash Buffer, connect the tube:

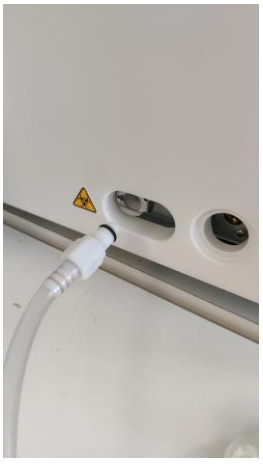
3.3.1.2 FILL THE DISTILLED WATER BOTTLE (0.25L CAPACITY)

Once the tank is filled with distilled water, connect the tubing:

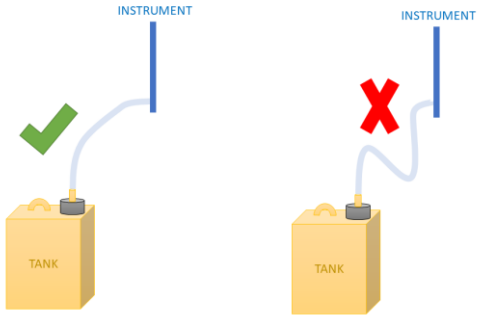
3.3.1.3 EMPTY THE WASTE TANK

Take the tube with quick connector

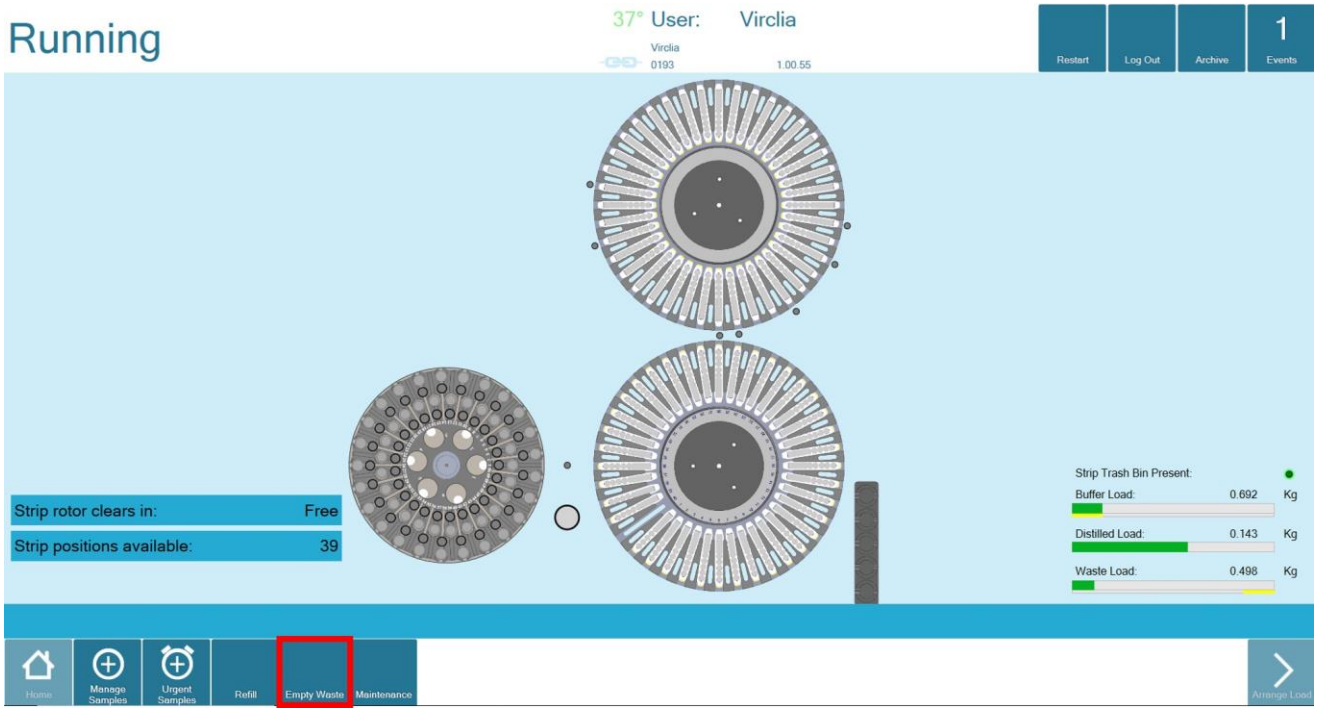
Connect to the connector on the left



Avoid any fold of the tubing to enable the correct flow of liquid:



Then, press the “Empty Waste” button, located on the lower taskbar and wait until the liquid stops to flow out from the instrument.



Vircell S.L. recommends to work with the auto empty waste function enabled (consult Technical Service for the correct configuration of this function) to avoid the manual emptying of the waste tank.

3.3.1.4 EMPTY THE STRIP WASTE CONTAINER

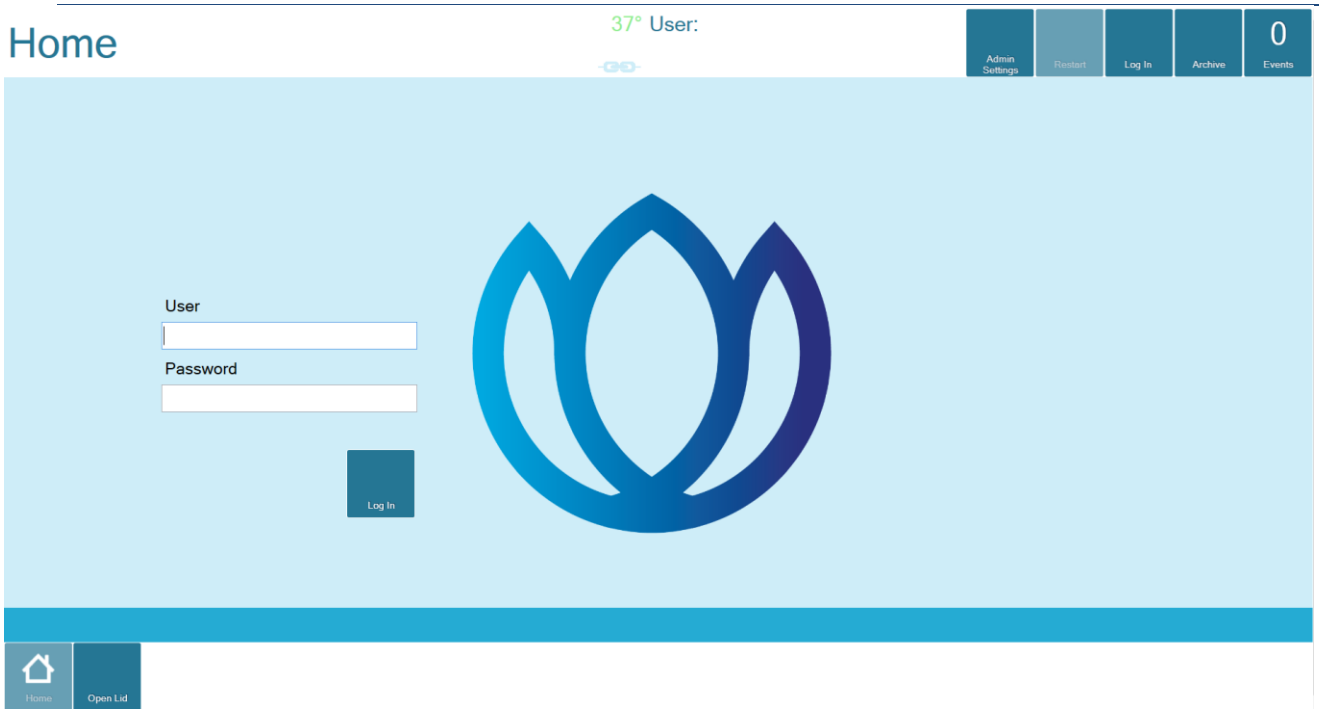
Every time new VirClia Strips are added, the software informs the user to make sure that the Strip Waste Container is empty.

To empty it, open the front white cover and take the Strip Waste Container out. Then return the container to its initial position.

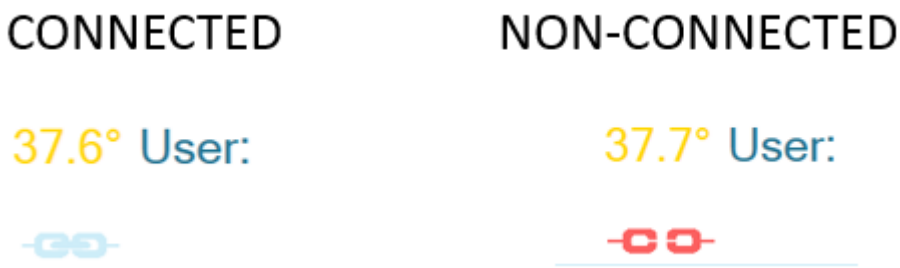
Make sure that the orientation of the Strip Waste Container is correct (please see figure below):

3.3.2 HOME SCREEN

Click on the VirClia Lotus™ icon, and the Lotus Application Program will open.

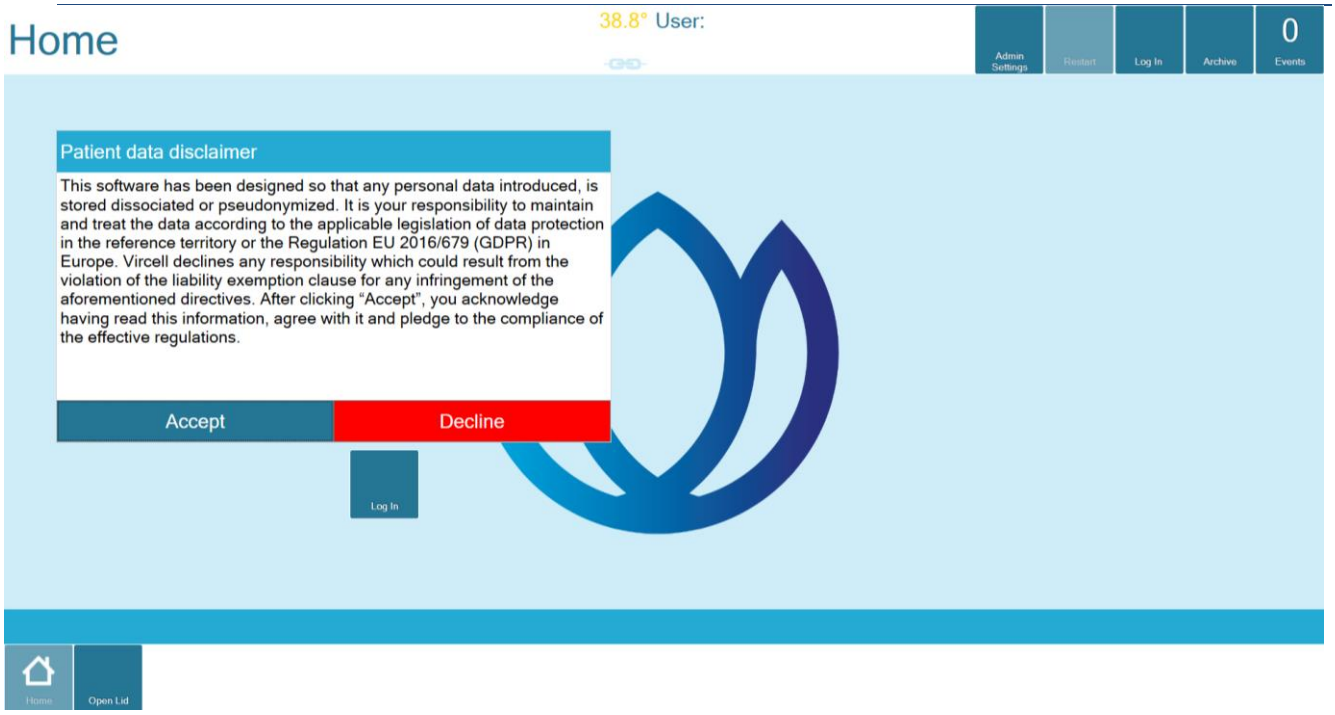


On the upper side of the screen, a message is shown informing if VirClia Lotus™ is able to establish connection with the computer. If VirClia Lotus™ is not connected to the computer, the chain icon is shown highlighted in red (see figure below).

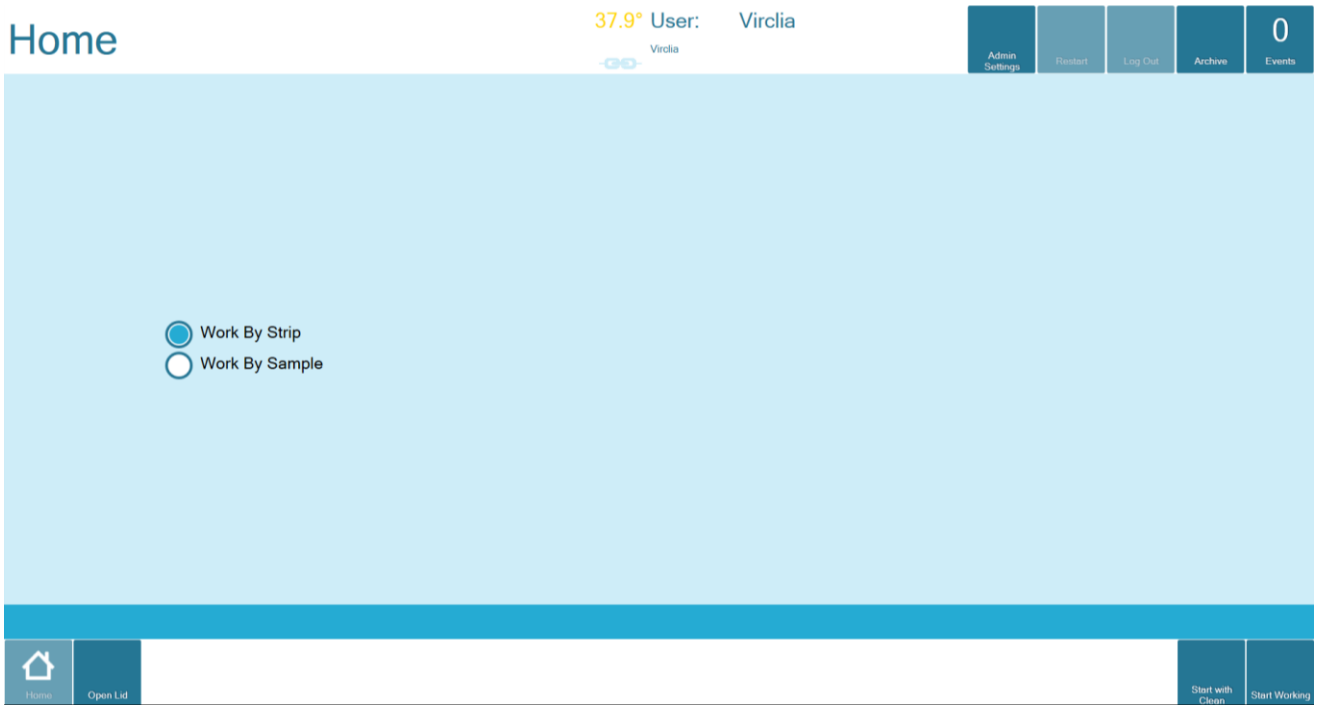


Enter the username and password and press *Log In* to continue. Should a user not be registered, the software will not allow to perform any action but opening the lid of VirClia Lotus™.

When a new user logs in the application for the first time, it is mandatory to accept the patient data disclaimer message which appears at this time. If the user declines it, log in process will not continue.



Once the disclaimer is accepted, the user can choose between "Work by Strip" or "Work by Sample". When "Work by Strips" is selected, the priority is given to those techniques with higher number of strips assigned to them. When "Work by Sample" is selected, the priority is given to those samples with higher number of strips associated. In both cases, the software informs the user how the strips must be loaded inside the strip rotor.

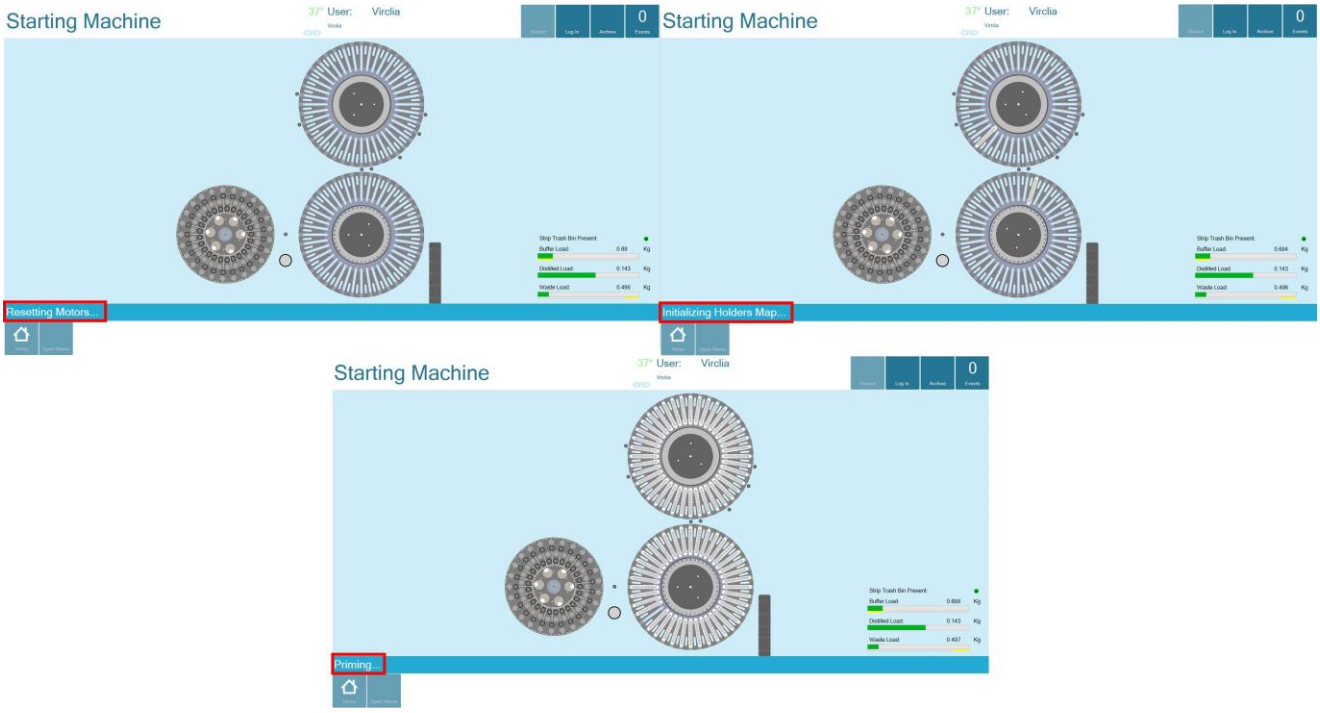


Two options for initializing the instrument are available: "Start with Clean" or "Start Working".

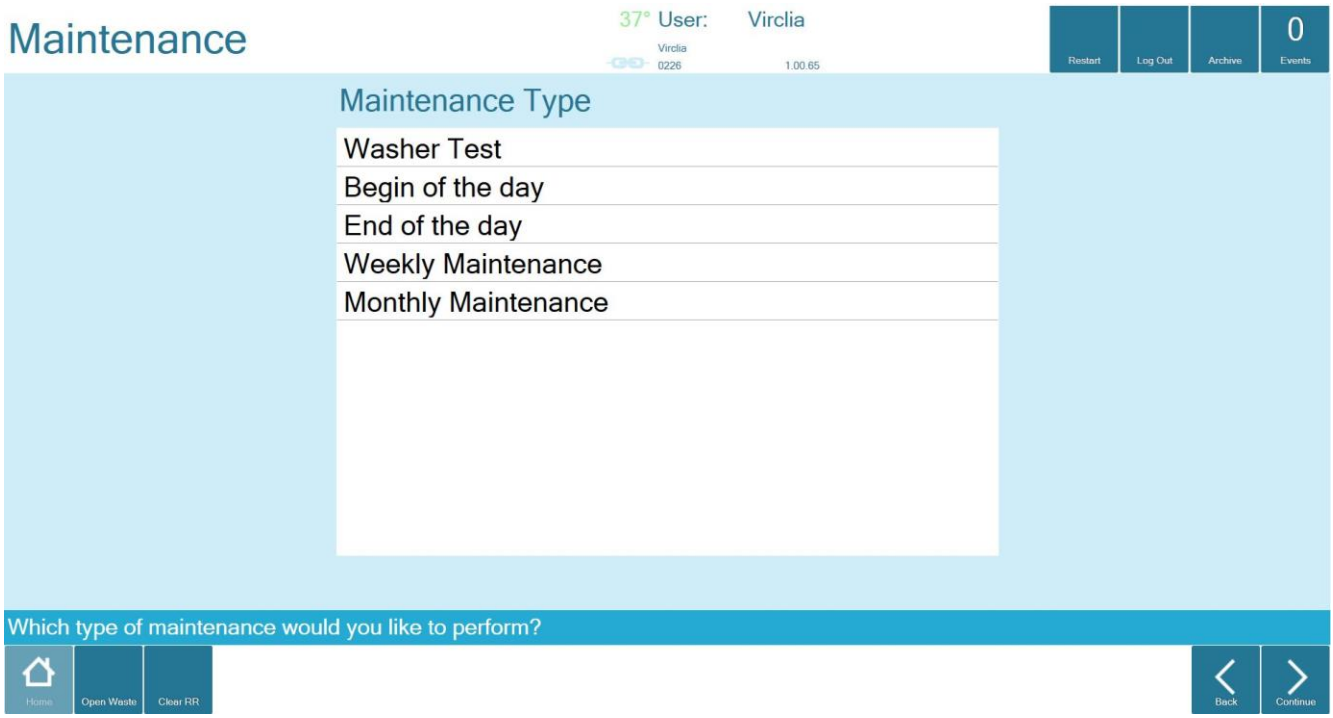
Initializing VirClia Lotus™ with the “Start Working” option means that the instrument will execute its initialization and perform a hydraulic priming with wash buffer.

Initializing VirClia Lotus™ with the “Start with Clean” option means that the instrument will execute its initialization, starting with a distilled water priming. The steps are as described below:

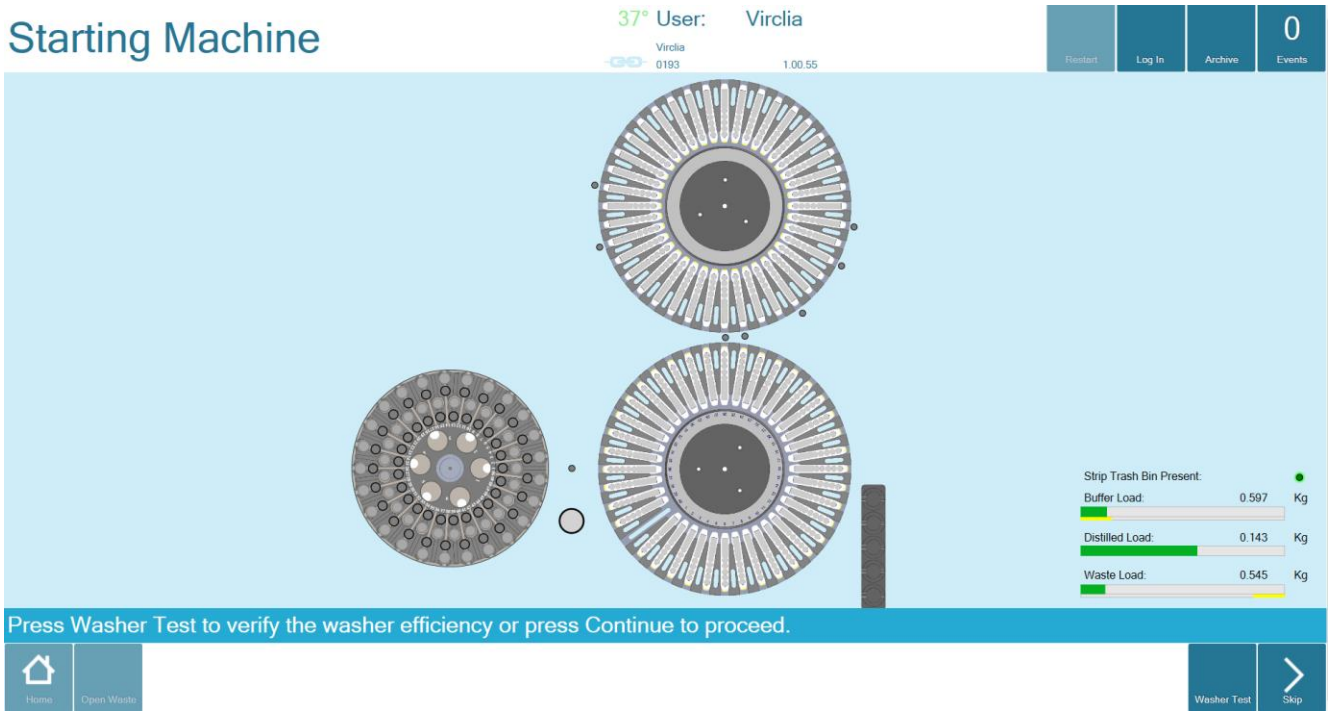
- Reset of all the moving parts to their home positions.
- The Strip Rotor and Reaction Rotor map the presence and position of all the strip holders and find the empty position.
- Cleaning the hydraulic circuit with distilled water (only if the option “Start with Clean” is selected).
- Hydraulic circuit priming to properly fill all the tubing, dilutors, pumps and needles with wash buffer.

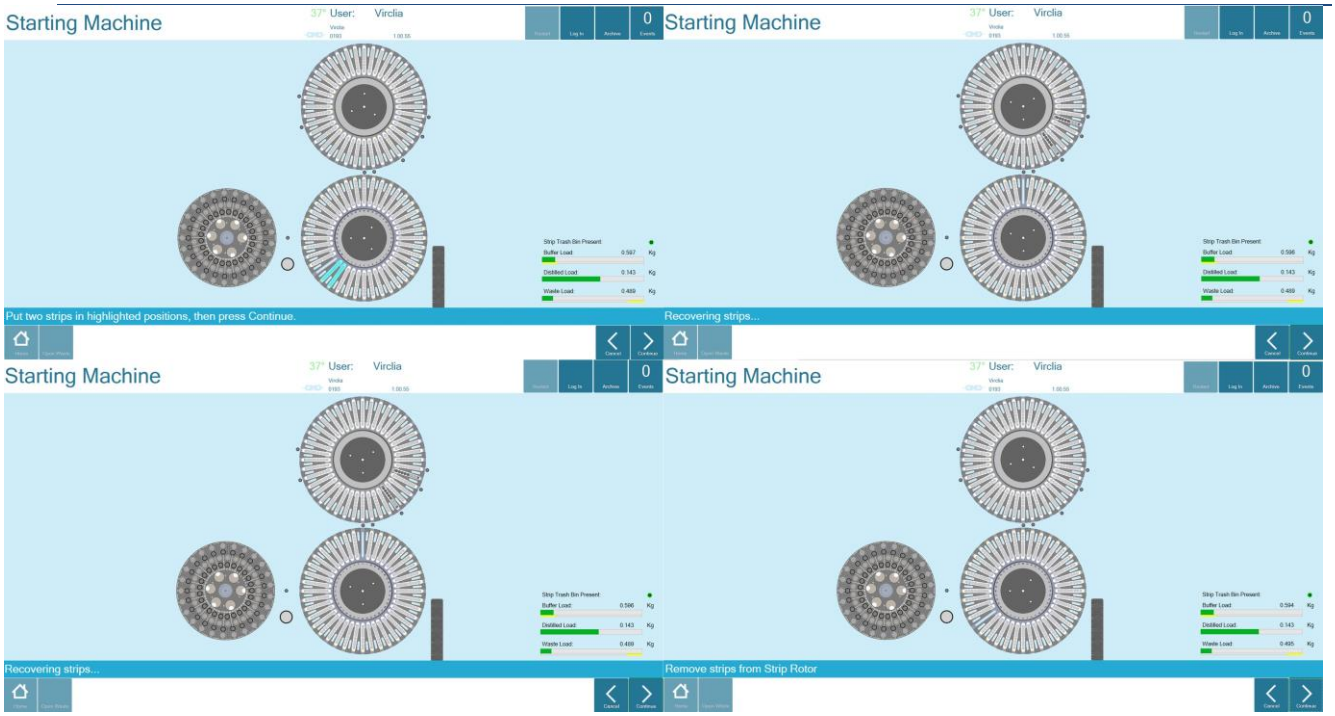


When the initialization finishes, the tab “Washer Test” is enabled. It is also possible to perform a washer test through the maintenance menu.



With the option “Washer Test” the instrument requests the operator to insert two strips inside the positions indicated by the software in the strip rotor. The strips are transferred to the reaction rotor. The software proceeds with the washing step for both strips, leaving one of them with the reaction wells empty; and the other one with the wells full of liquid. Finally, the strips are transferred back to the Strip Rotor, allowing the operator to recover them in order to check if the strip washer is working properly. Selecting the option “Skip”, the user can continue without performing this verification test for the washer.



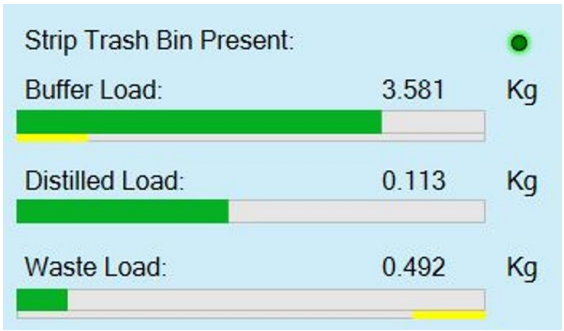


By pressing the "Continue" button, the instrument is ready for starting the assay and the "Running" screen is displayed. Refer to Section 3.3.1 which contains the instructions on how to load the wash buffer and the distilled water, as well as for emptying the strip waste container and the waste tank, if needed.

3.3.3 RUNNING SCREEN (PART 1)

On the main screen, a schematic representation of the instrument layout is displayed. On the left side of the screen, the sample rotor is shown. On the right side, the strip rotor and the reaction rotor are displayed. Finally, on the right side of the strip rotor, the reagent rack is displayed.

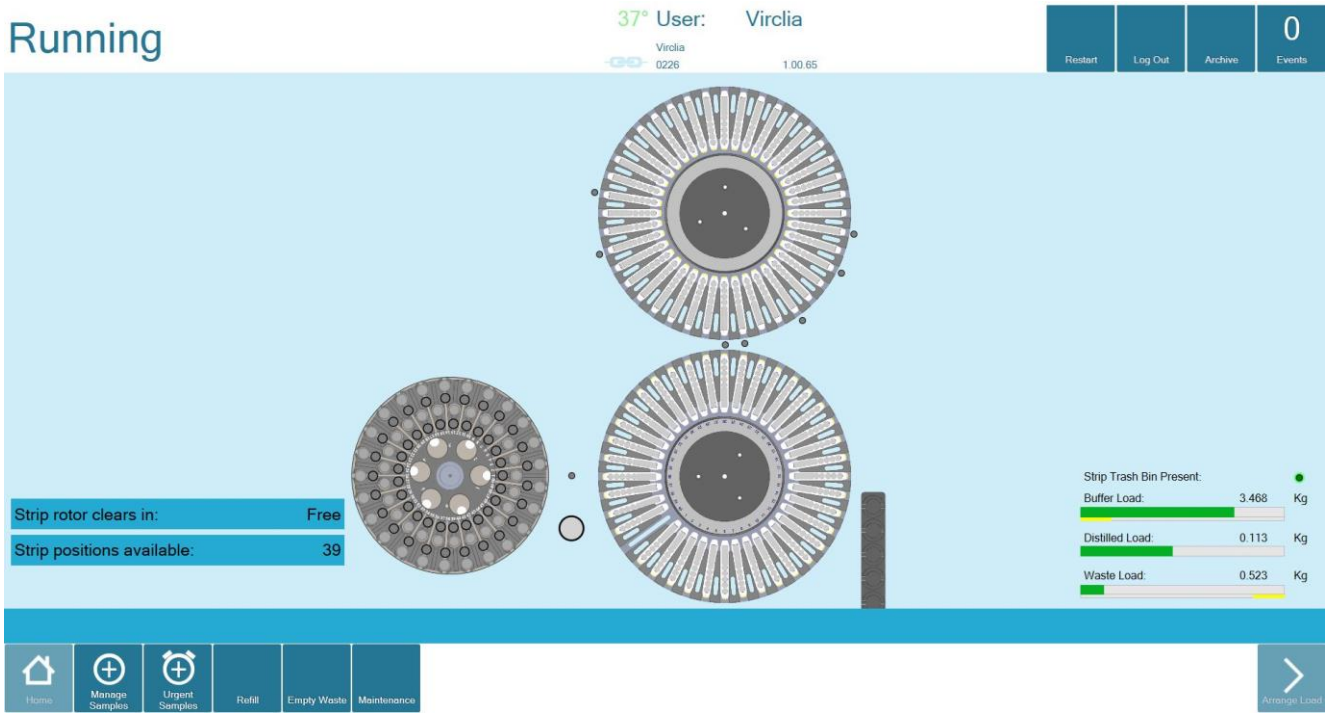
In addition, the software shows information related to the status of the Strip Waste Container (green, yellow or red), and the levels of the Waste, Wash Buffer and Distilled Water.



The bottom yellow bar below the wash buffer is the expected wash buffer consumption for the strips already loaded, and it always needs to be shorter than the green bar. If starting a new assay would result in the yellow bar being larger than the

green one, the new assay will not be able to be processed until the wash buffer tank is refilled, and the green bar is larger than the yellow one.

For the waste tank, it is similar, but shows required waste space.



The “Running” screen shows additional information:

- **Strip rotor clears in:** It indicates the time needed for an available space within the strip rotor. The status shown as “free” means that the strip rotor is empty.
- **Strip positions available:** It indicates the number of positions available in the strip rotor.

In the bottom left side of the screen some tabs are displayed to allow the user to:

- **“Manage Samples”:** Add new samples.
- **“Urgent Samples”:** Add urgent samples.
- **“Refill”:** Refill the Wash buffer tank and/or reagents.
- **“Empty Waste”:** Empty the waste tank.
- **“Maintenance”:** To select which maintenance is going to be performed (begin of the day maintenance, end of the day maintenance, weekly maintenance or monthly maintenance).
- **“Arrange Load”:** Allows the user to load more strips during an assay when the instrument asks for more resources.

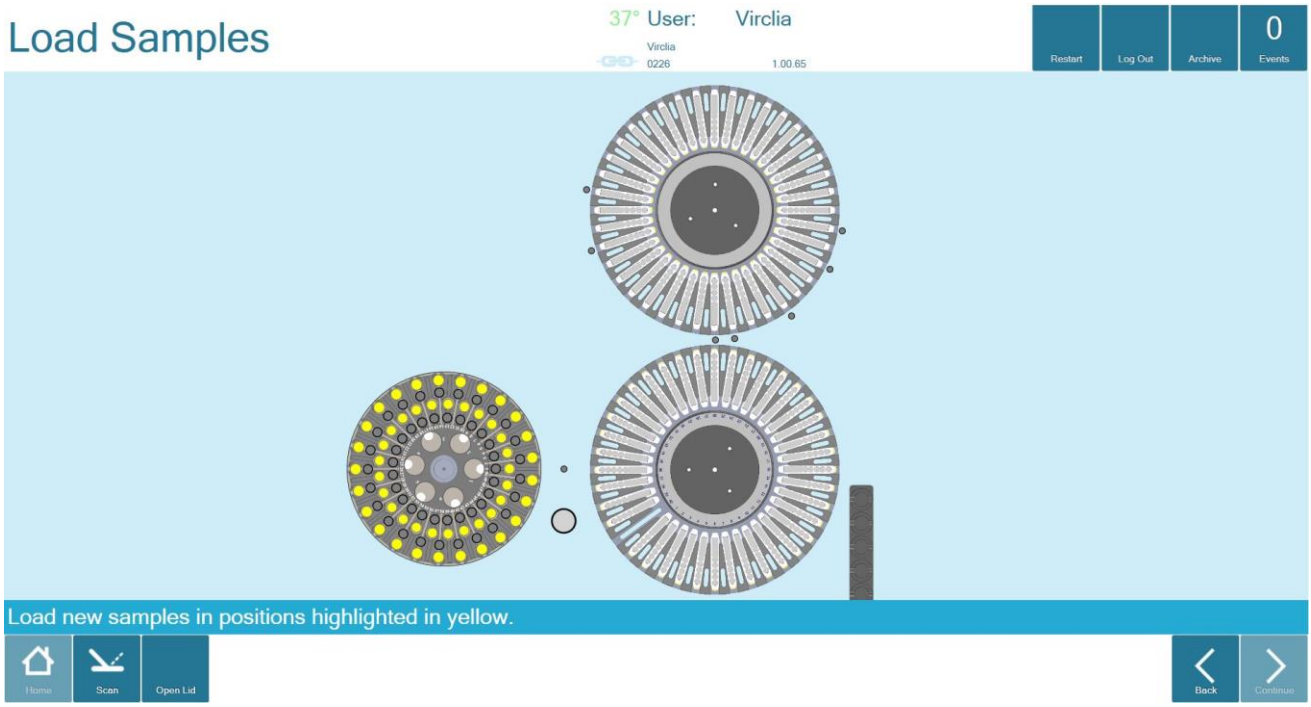
In the upper right section of the screen, the software displays the following tabs:

- **“Restart”:** Restarts the instrument.
- **“Log out”:** User can close the session.
- **“Archive”:** For reviewing the results.
- **“Events”:** For checking instrument anomalies, log file and maintenance report generation.

To begin with the run, click “Add Samples”.

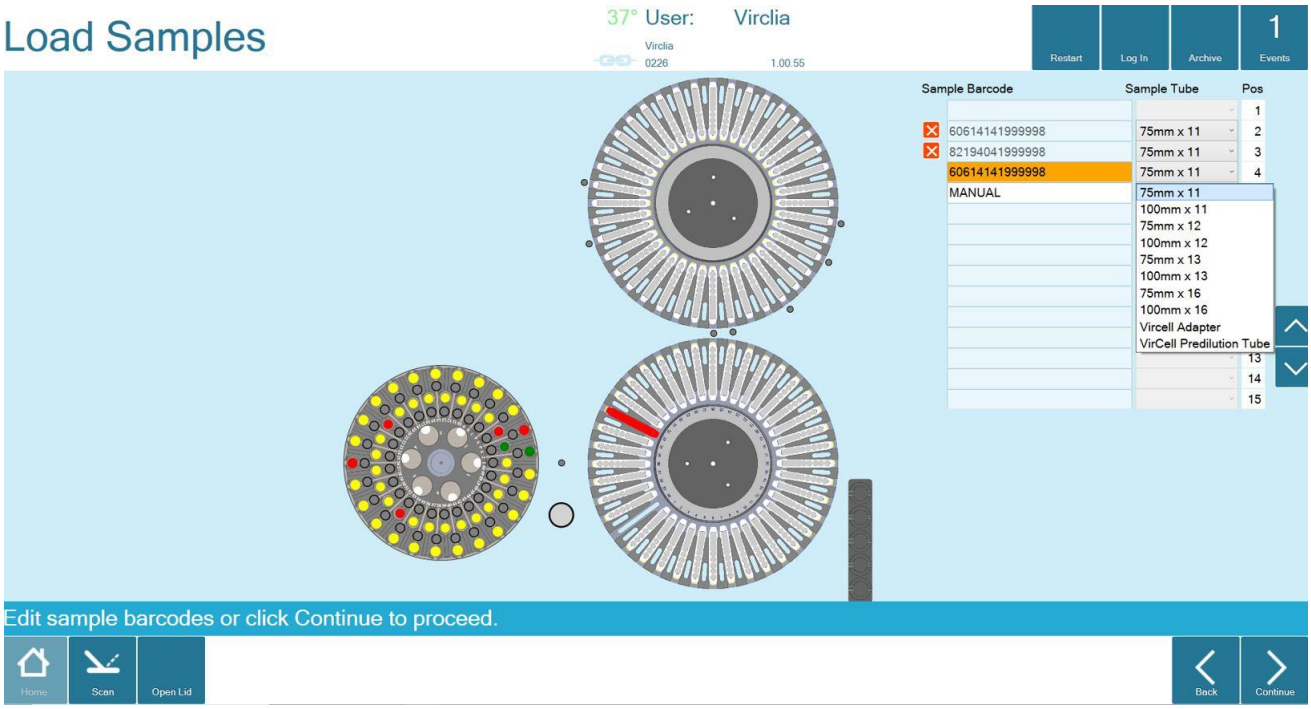
3.3.4 LOAD SAMPLES SCREEN

The “Load Samples” screen shows in yellow the free positions available in the Sample Rotor where the sample tubes can be placed.



Insert the sample tubes within any of the available positions with the barcode identification label facing outward from the rotor (see picture below).

Click on the “Scan” button to automatically scan the samples.
Once the automatic reading is executed, a list with all the codes is shown. These samples can be deleted by clicking on the “X” box (see below). In the event that any label is not readable or the sample tube has no barcode, the corresponding field position will be editable so the user can manually add the sample ID. Dimensions for each tube must be selected using the drop-down menu of each row. Lotus Application will highlight the repeated sample tube codes in orange.



Empty positions are detected by means of a background barcode label in the inner part of the sample rotor. When there is a tube in between the barcode reader and the background barcode, the barcode reader is not able to read it and the software detects that the position is occupied. Otherwise, the position is shown as empty, and highlighted in light blue on the sample list and the corresponding field is not editable.

The user can scroll up and down through the 50 positions in the list by pressing the corresponding buttons. Once the sample list is filled in correctly, the user can proceed by pressing the “Continue” button.

There are three ways of identifying the sample tubes. This can be configured through the Service mode (refer to your local distributor for this matter):

- One shot attempt: All the positions available are scanned in one single turn of the sample rotor.
- One sample at a time: All the positions are scanned individually. Those positions with unreadable barcode or empty are rescanned in the same turn of the sample rotor.
- Retry unreadable positions: All the positions of the rotor are scanned in a first turn of the sample rotor. Those positions occupied which have unreadable barcode are scanned in a second turn of the sample rotor.

Manual identification of the samples is limited to certain characters admitted for the sample identification. Characters admitted are summarized in table below. Space and underscore symbols should not be used.

Symbol	Description
!	Exclamation mark
"	Double quotes (or speech marks)
#	Number
$	Dollar
%	Per cent sign
&	Ampersand
'	Single quote
(	Open parenthesis (or open bracket)
)	Close parenthesis (or close bracket)
*	Asterisk
+	Plus
,	Comma
-	Hyphen
.	Period, dot or full stop
/	Slash or divide
0	Zero
1	One
2	Two
3	Three
4	Four
5	Five
6	Six
7	Seven
8	Eight
9	Nine
:	Colon
;	Semicolon
<	Less than (or open angled bracket)
=	Equals
>	Greater than (or close angled bracket)
?	Question mark

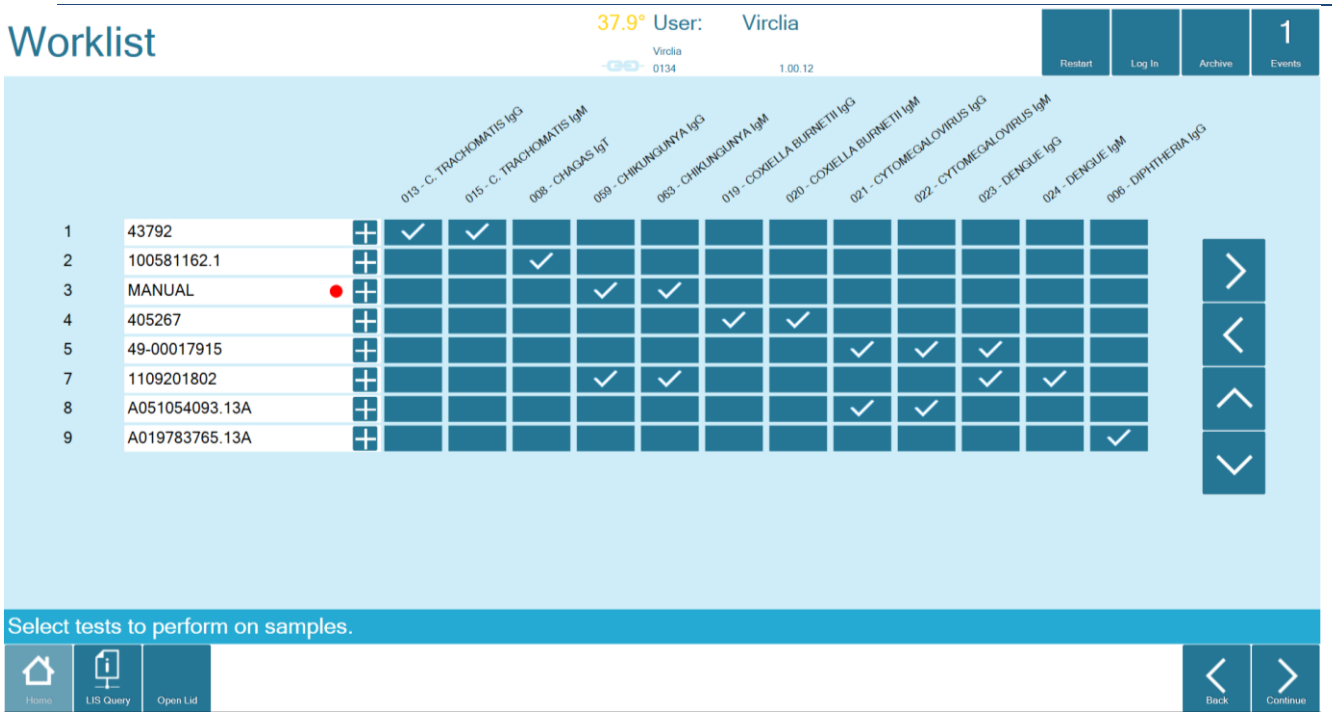
Symbol	Description
@	At symbol
A	Uppercase A
B	Uppercase B
C	Uppercase C
D	Uppercase D
E	Uppercase E
F	Uppercase F
G	Uppercase G
H	Uppercase H
I	Uppercase I
J	Uppercase J
K	Uppercase K
L	Uppercase L
M	Uppercase M
N	Uppercase N
O	Uppercase O
P	Uppercase P
Q	Uppercase Q
R	Uppercase R
S	Uppercase S
T	Uppercase T
U	Uppercase U
V	Uppercase V
W	Uppercase W
X	Uppercase X
Y	Uppercase Y
Z	Uppercase Z
[	Opening bracket
\	Backslash
]	Closing bracket
^	Caret - circumflex

Symbol	Description
`	Grave accent
a	Lowercase a
b	Lowercase b
c	Lowercase c
d	Lowercase d
e	Lowercase e
f	Lowercase f
g	Lowercase g
h	Lowercase h
i	Lowercase i
j	Lowercase j
k	Lowercase k
l	Lowercase l
m	Lowercase m
n	Lowercase n
o	Lowercase o
p	Lowercase p
q	Lowercase q
r	Lowercase r
s	Lowercase s
t	Lowercase t
u	Lowercase u
v	Lowercase v
w	Lowercase w
x	Lowercase x
y	Lowercase y
z	Lowercase z
{	Opening brace
|	Vertical bar
}	Closing brace
~	Equivalency sign

3.3.5 WORKLIST SCREEN

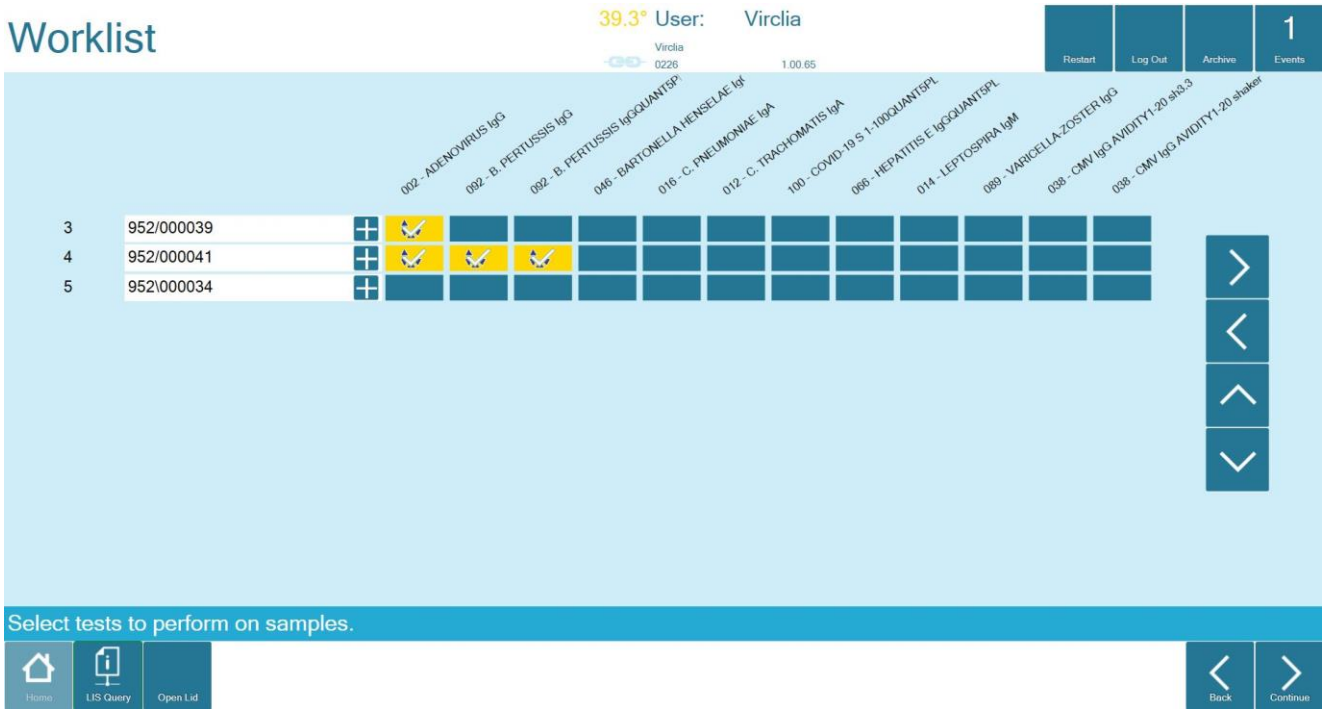
The “Worklist” screen allows assigning one or more assays to each sample. A red dot next to the sample ID means that the ID has been manually input. Navigate through the samples and tests list using the arrow buttons.
This step can be performed in two modes: manually or automatically using “LIS Query” button (if the LIS communications have already been established).

Manually: click on the box of the corresponding column of the test to be selected.



Replicates of the loaded samples can be added clicking in the button twice:

LIS Query: click on the “LIS Query” button to request the Worklist from the laboratory host computer. The Worklist received can be manually edited, if necessary.



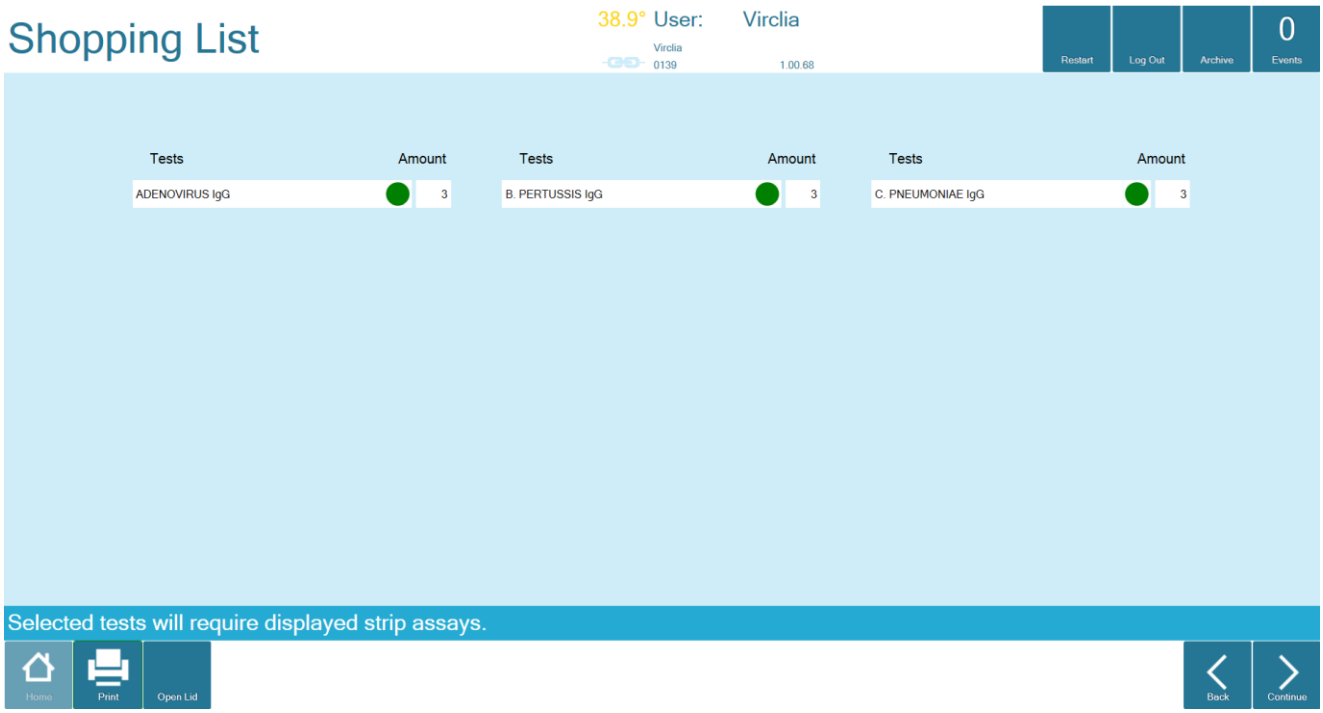
The icon  shows tests assigned by the LIS.

By clicking on the (+) button twice (this button is close to the sample ID code), the user can run replicas of the samples.

Once the Worklist has been created, press “Continue” to proceed.

3.3.6 SHOPPING LIST SCREEN

The software shows a “Shopping List”, that is, a list including all the VirClia Strips needed to run the analysis session, grouped by type of test. In this way, the user can know and print, if necessary, the type and quantity of VirClia Strips required for the run.

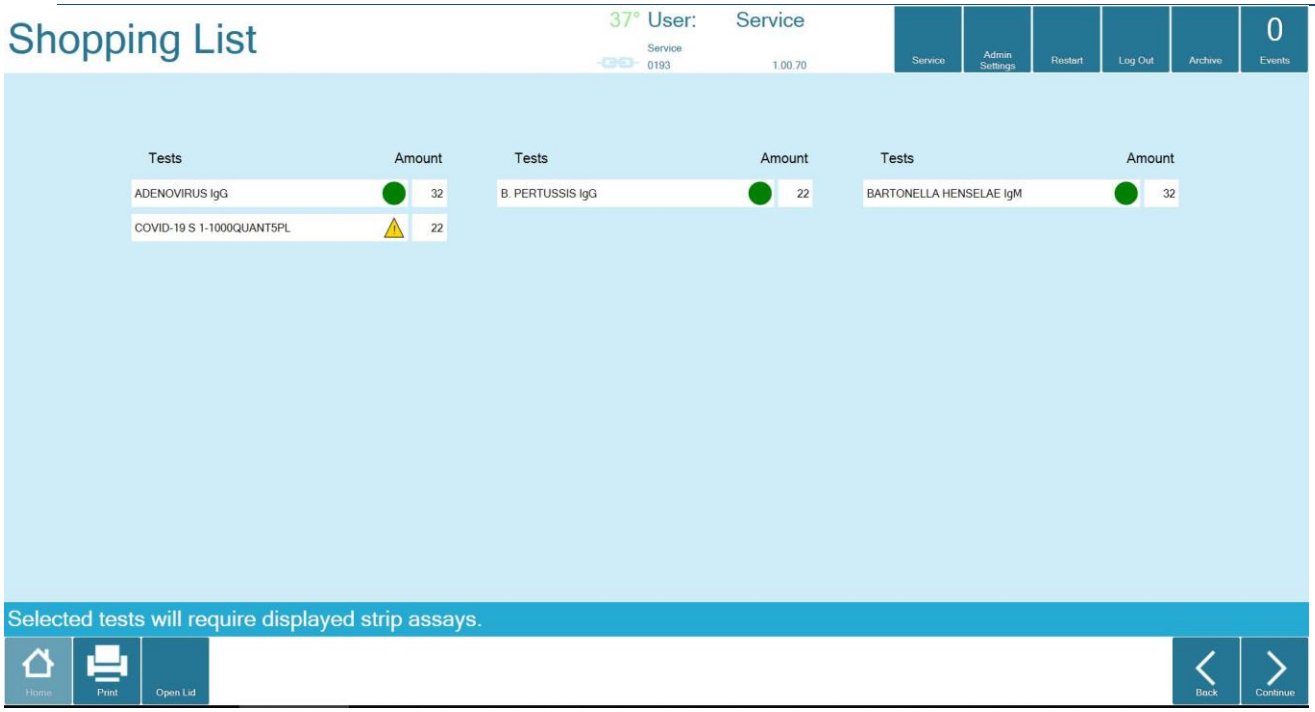


Click on "Print" to generate a PDF file that summarizes the number of strips required for the assay.

VirClia Lotus
Tuesday, August 30, 2022 5:32:27 PM
Instrument ID: 2019-05-0139 User: Virclia

Tests	Amount
ADENOVIRUS IgG	3
B. PERTUSSIS IgG	3
C. PNEUMONIAE IgG	3

If a technique appears with this alert symbol  it means that 5PL run time variable will need to be typed. Those techniques which appear with a green dot will not require to type any run time variables.

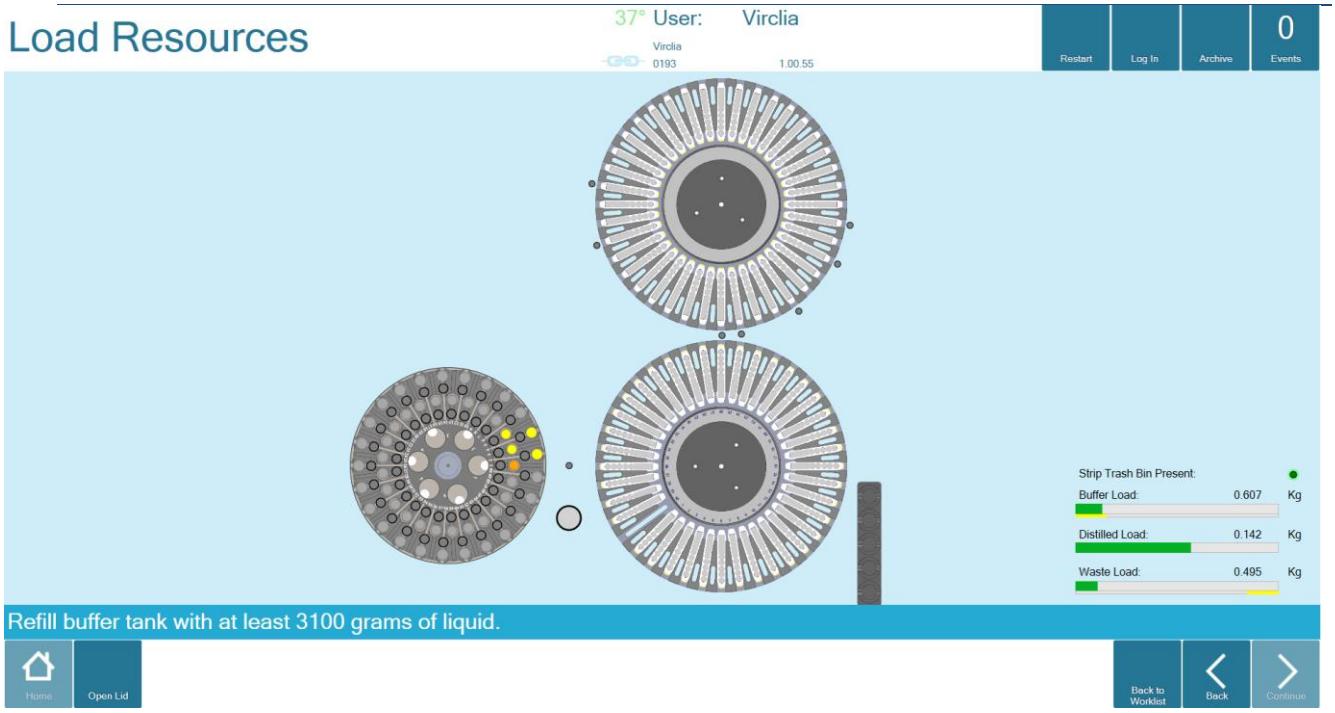


Press “Continue” to proceed.

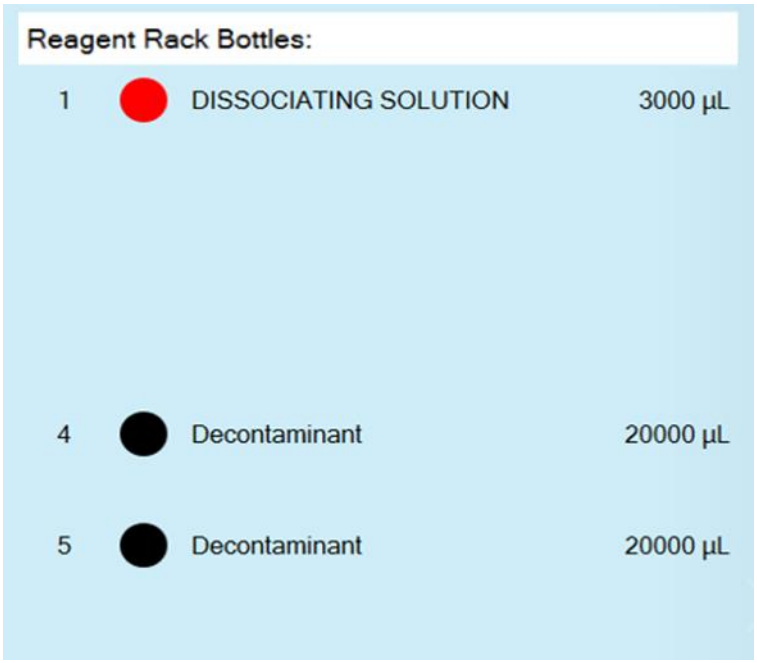
3.3.7 LOAD RESOURCES SCREEN

In this sequence of screens, the user is required to perform some verifications prior to start the run. He is also requested to load additional resources.

If the instrument detects that there is not enough wash buffer solution, the software will request to load the sufficient amount of reagent to perform the assay.



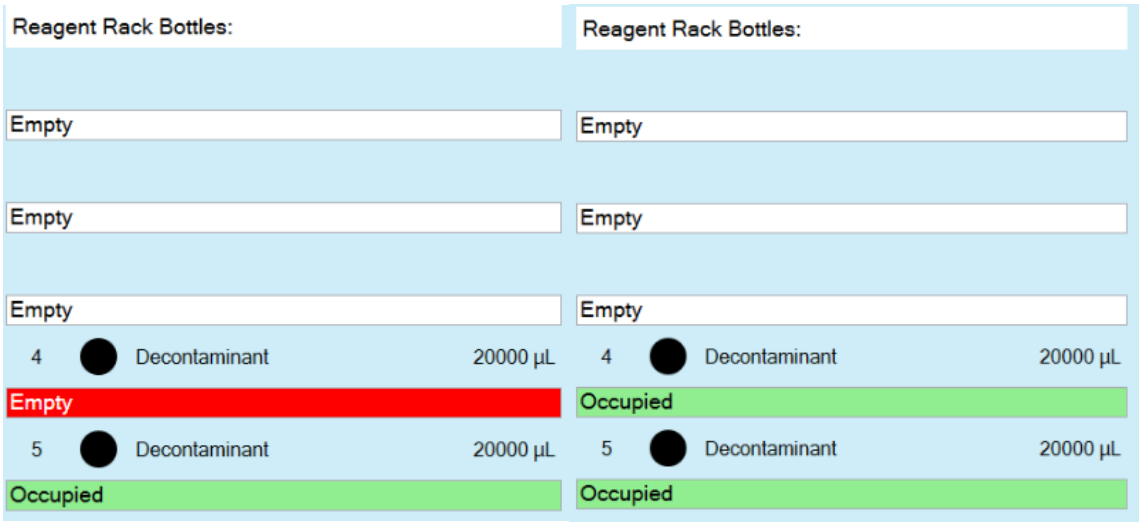
Decontaminant and additional reagent bottles shall be placed onto the auxiliary reagent rack located on the right side of the instrument in the indicated positions by the software, so that the instrument can scan the bottles:



Note that the nominal volume of the reagent bottle to be loaded is indicated on the right side of the reagent bottle name.

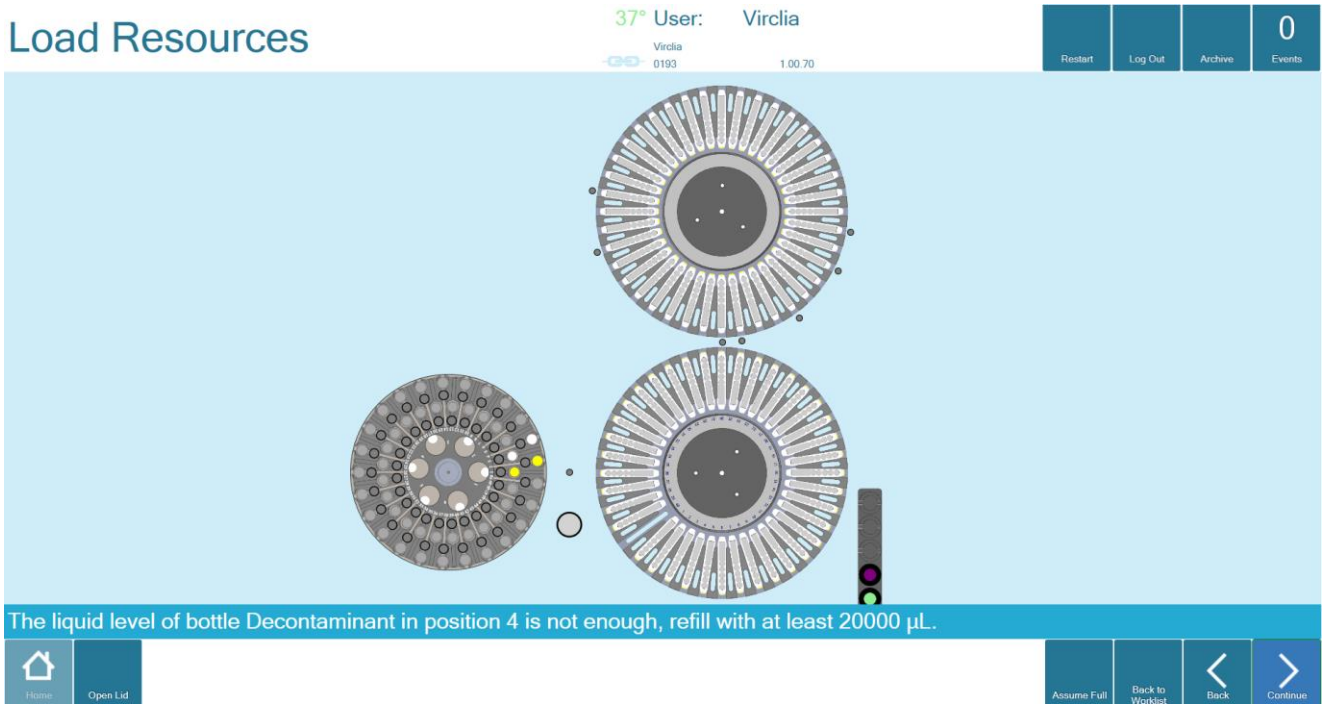
Press “Continue” to proceed.

If any of the reagent bottles is not placed in the indicated positions by the software inside the reagent rack, the user is informed that the position is empty. VirClia Lotus™ does not proceed with the assay until the required reagents are correctly placed. The reagent rack contains a background barcode to detect empty positions.

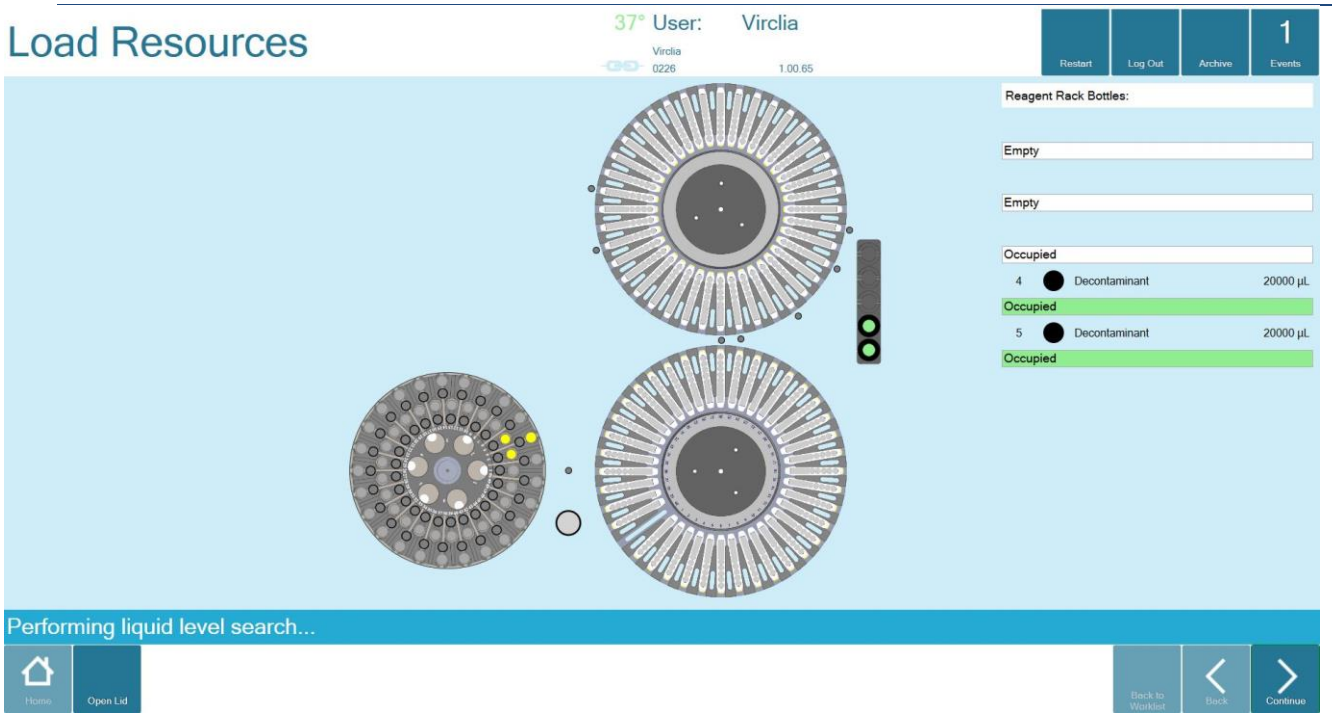


Click on the “Continue” button to proceed.

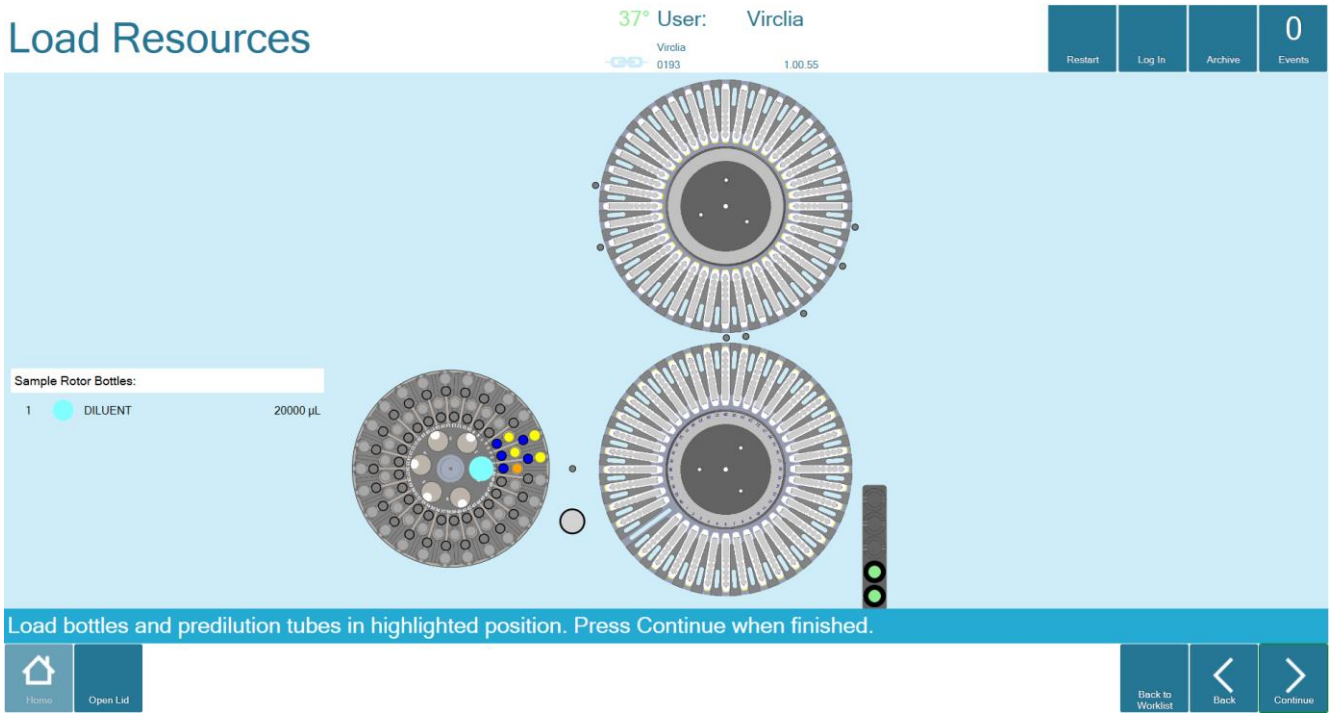
If all the requested reagent bottles were located in the indicated positions, a liquid level detection is performed in each one of them. If the volume of any of the bottles is not sufficient, the software will not allow to start the assay unless the sufficient volume is refilled in the indicated bottles.



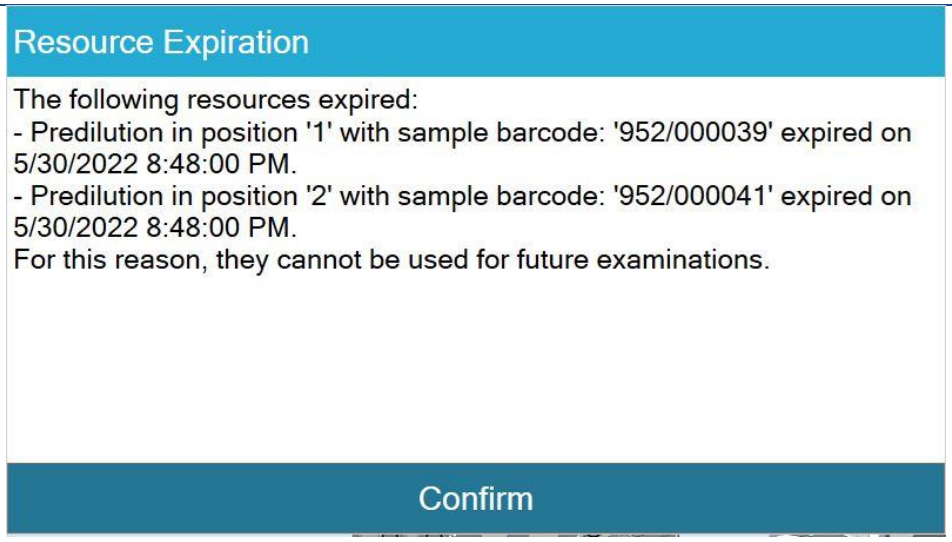
After refilling the sufficient amount of reagent, press continue to perform the liquid level detection again.



If the worklist contains a test requiring a sample predilution, the specific resources are requested in the next screen. EDP diluent bottles are represented in dark blue. This reagent is required for analyzing plasma samples with the plasma methods. EDB diluent bottles are represented in light blue. This reagent is required for analyzing serum samples with serum methods.

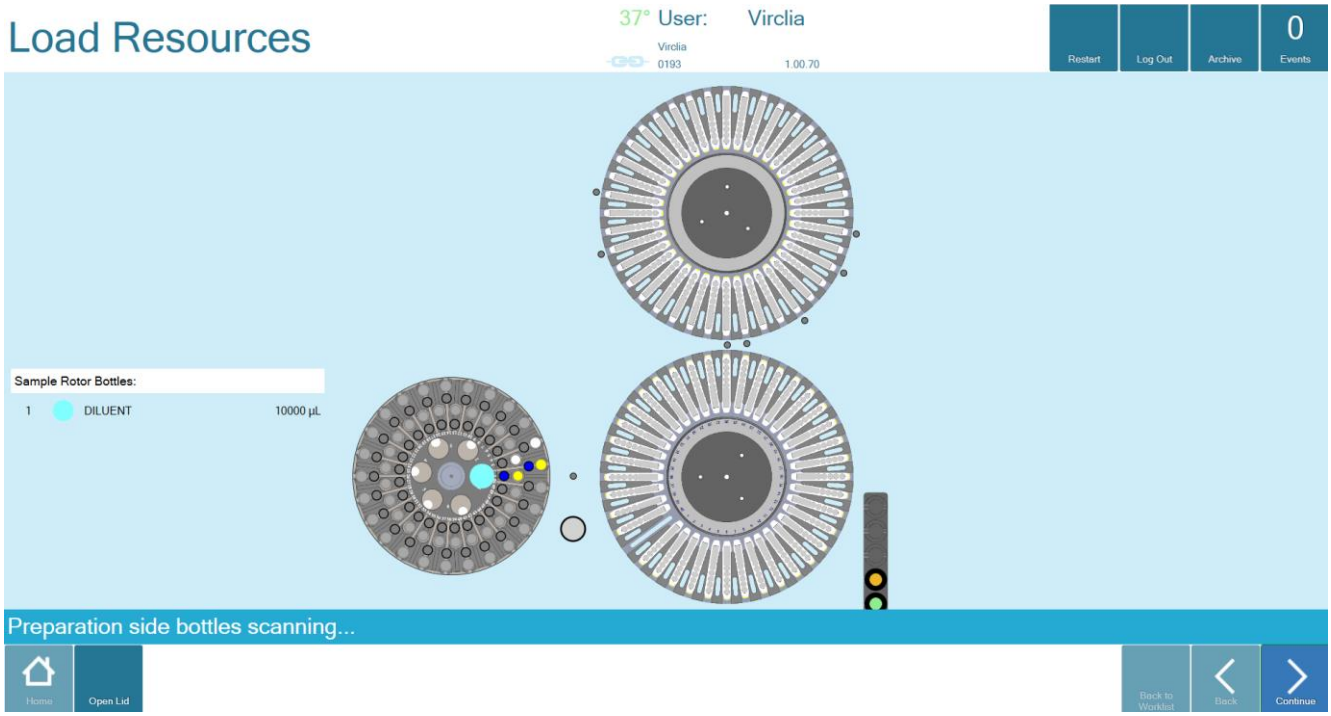


User has to bear in mind that the predilutions have an expiration time. The default expiration time is 180 minutes after sample is prediluted. If any prediluted sample exceeds this time in the sample rotor, the following pop up message will appear:

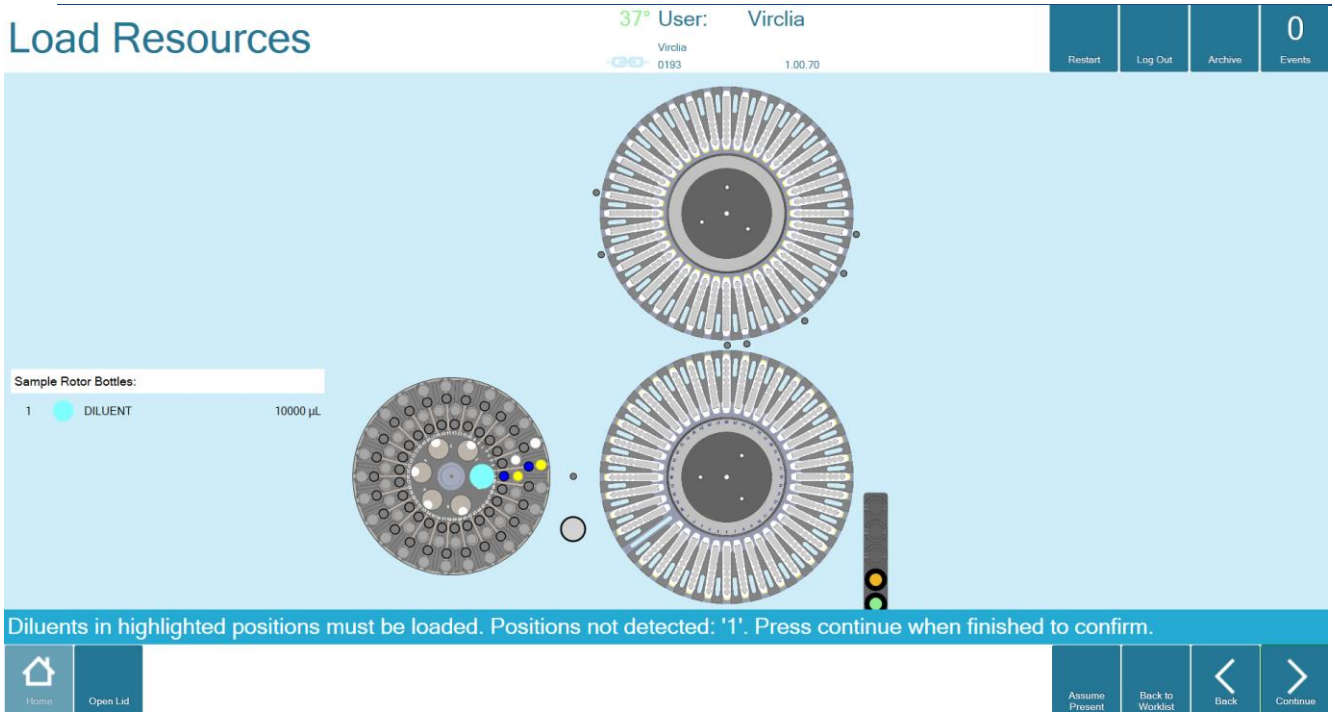


The software shows where to place diluent bottles (light blue), and predilution tubes (dark blue) within the sample rotor. The positions for the predilution tubes are independent from the sample positions and will start from position 1.

If the diluent bottles have been placed in the indicated positions, the instrument performs a detection of their presence.

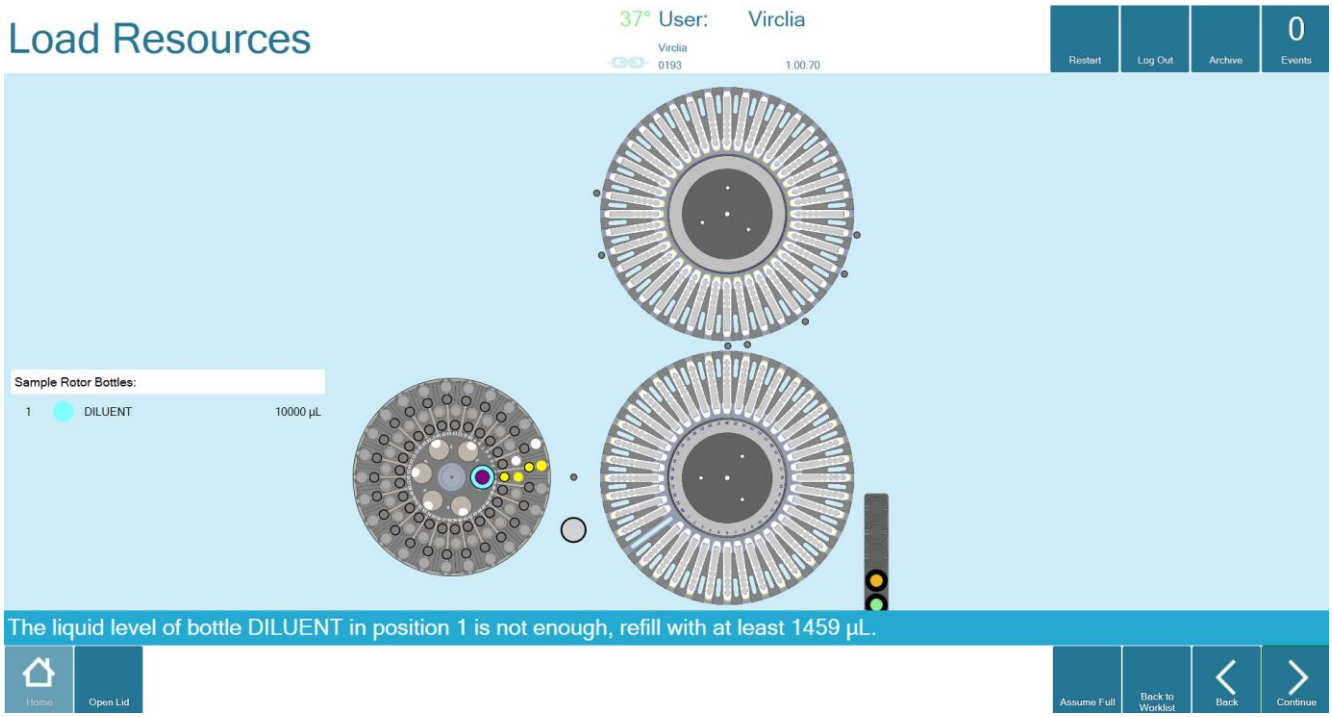


If any of the indicated positions is empty, the following message will be displayed.



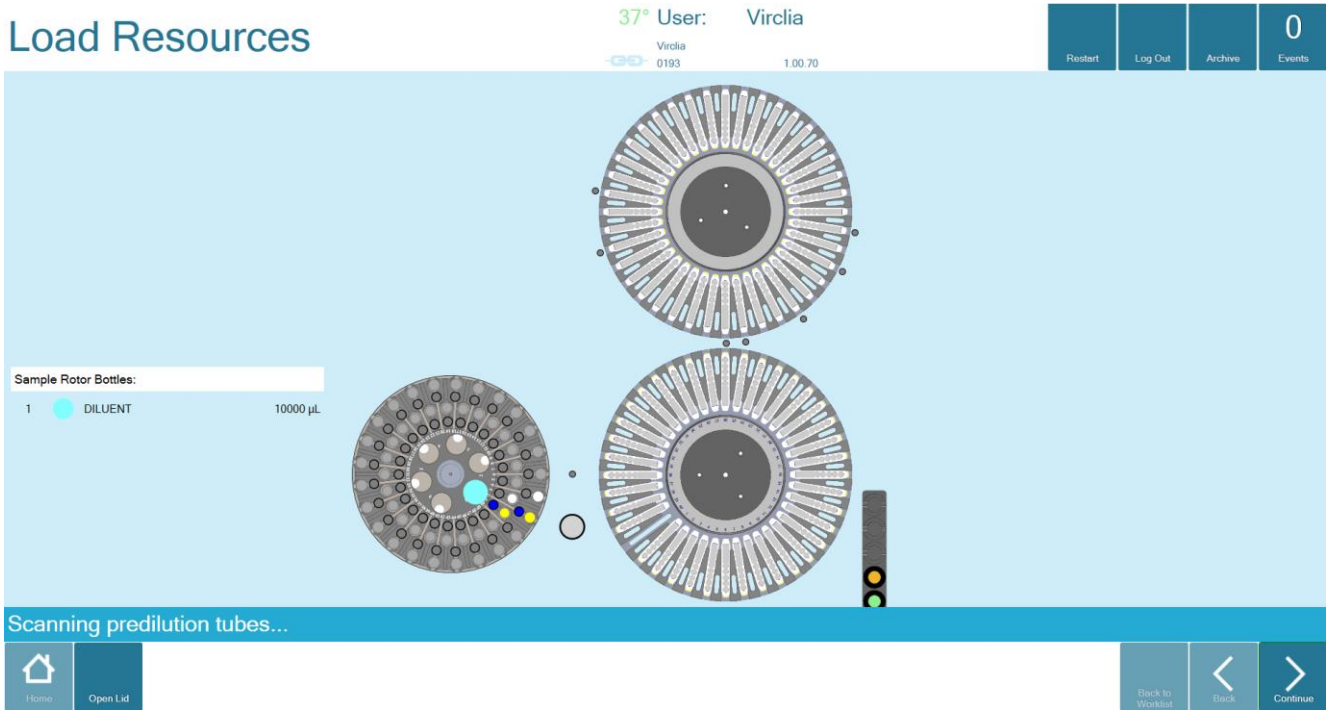
It is also possible to assume the presence of the bottle by means of the “Assume presence” button.

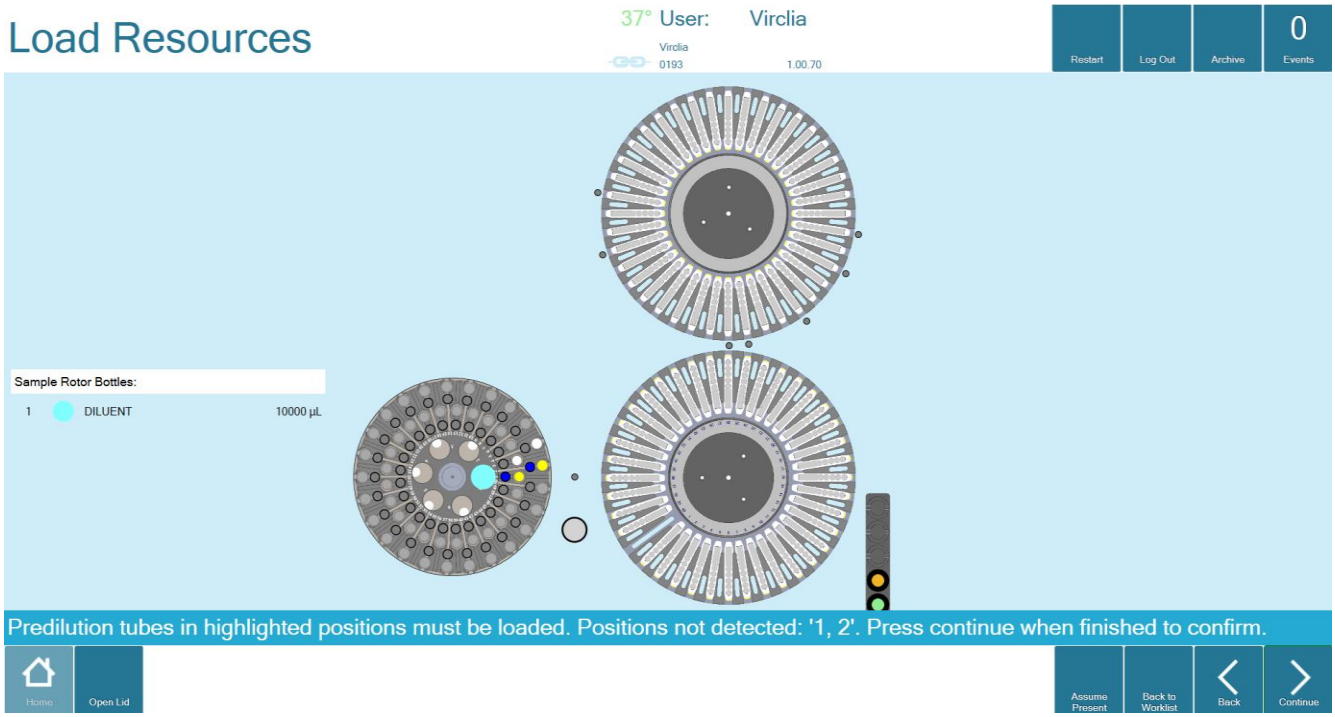
If all the bottles were correctly allocated in the indicated positions, the instrument will perform a liquid level detection to check the available volume. If the volume is not sufficient, the assay will not start unless the bottle is refilled with the requested amount of reagent.



After refilling the sufficient amount of reagent, press continue to perform again the liquid level detection. It is possible to assume that the bottles are full of liquid by means of the button “Assume full”.

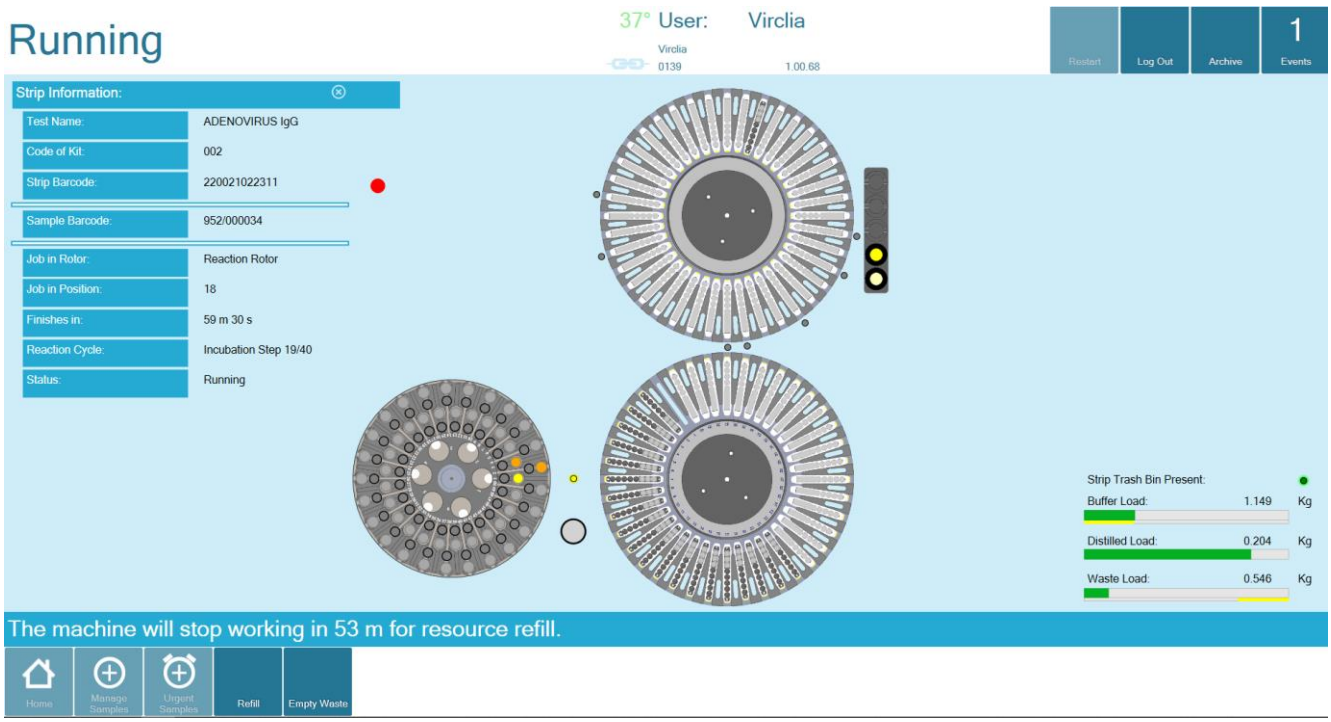
The software highlights in dark blue the positions were the predilution tubes should be placed. After loading the predilution tubes, the instrument performs a detection of the occupied positions. If a predilution tube is not detected, it will not be possible to start the assay unless the user loads the tubes in the requested positions.

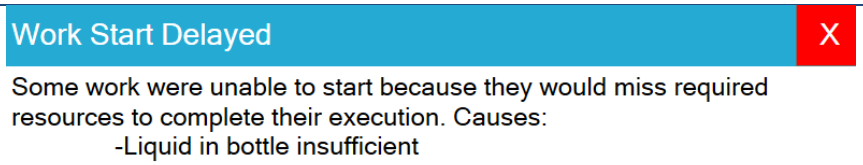


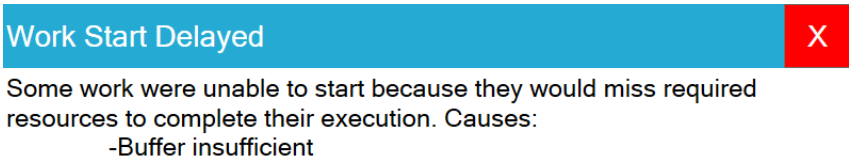


Press “Continue” to proceed.

If during a running assay new samples and/or assays are added, it is possible that the instrument does not have the sufficient resources to complete the new assays. The instrument will enter in a programmed stop and the software will indicate the required resources to complete the new assays.





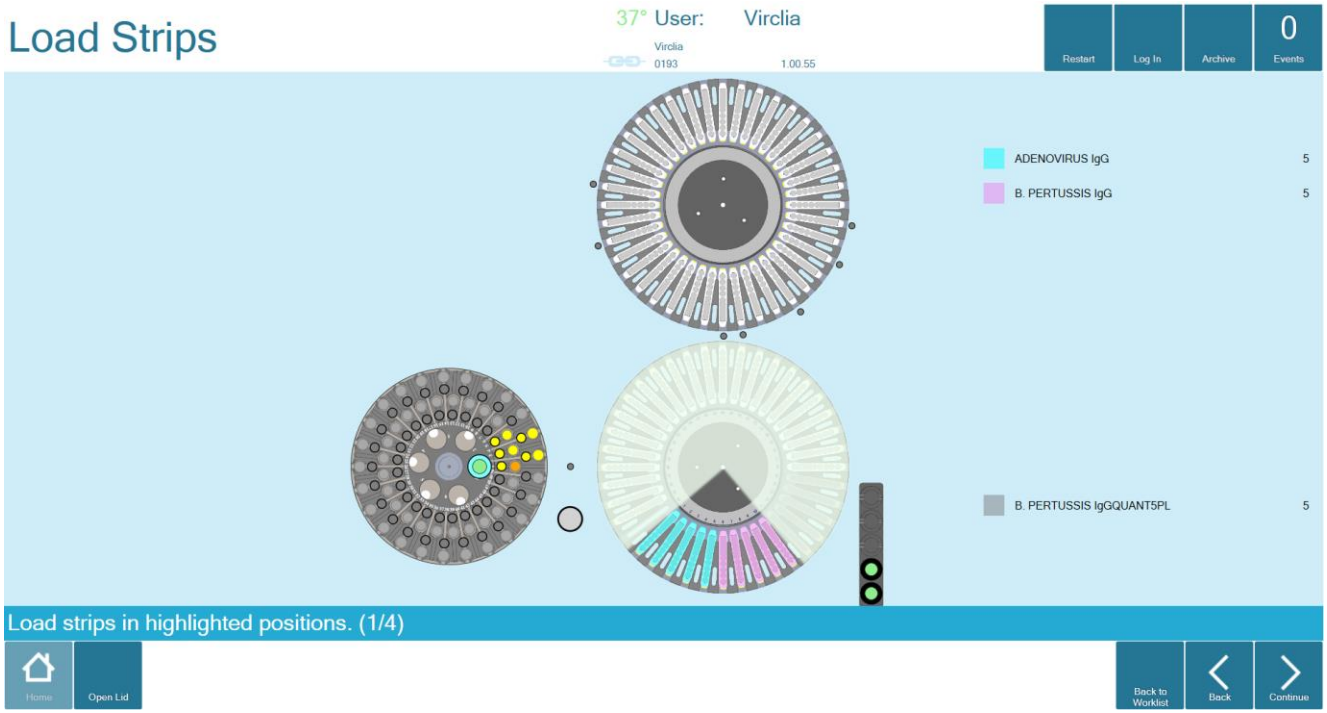


During a running assay there are two ways of loading more resources in the instrument. The first option is to allow the programmed stop time counter reach zero.

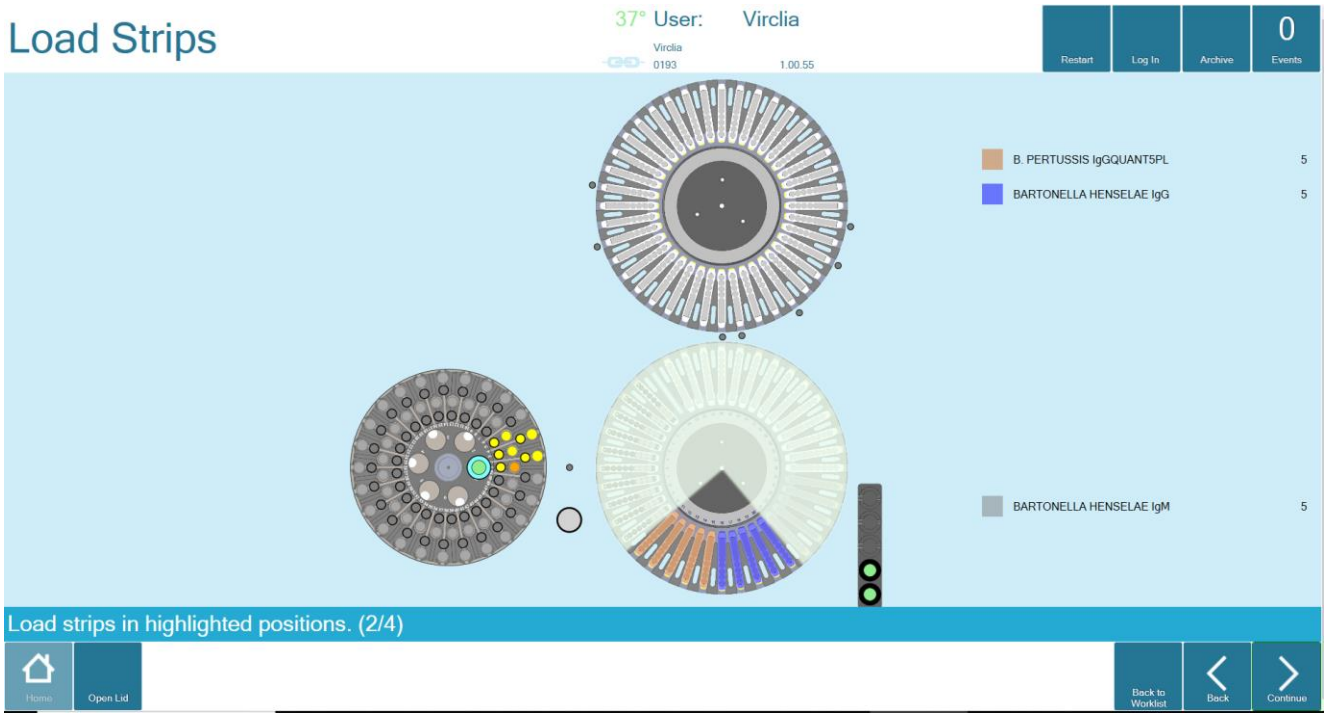
Another possibility is to load the resources while the assay is running by means of the "Refill" button. In this way, it is possible to skip the programmed stop. **Refer to section 3.3.14 "Resources loading during an assay" for more detailed information.**

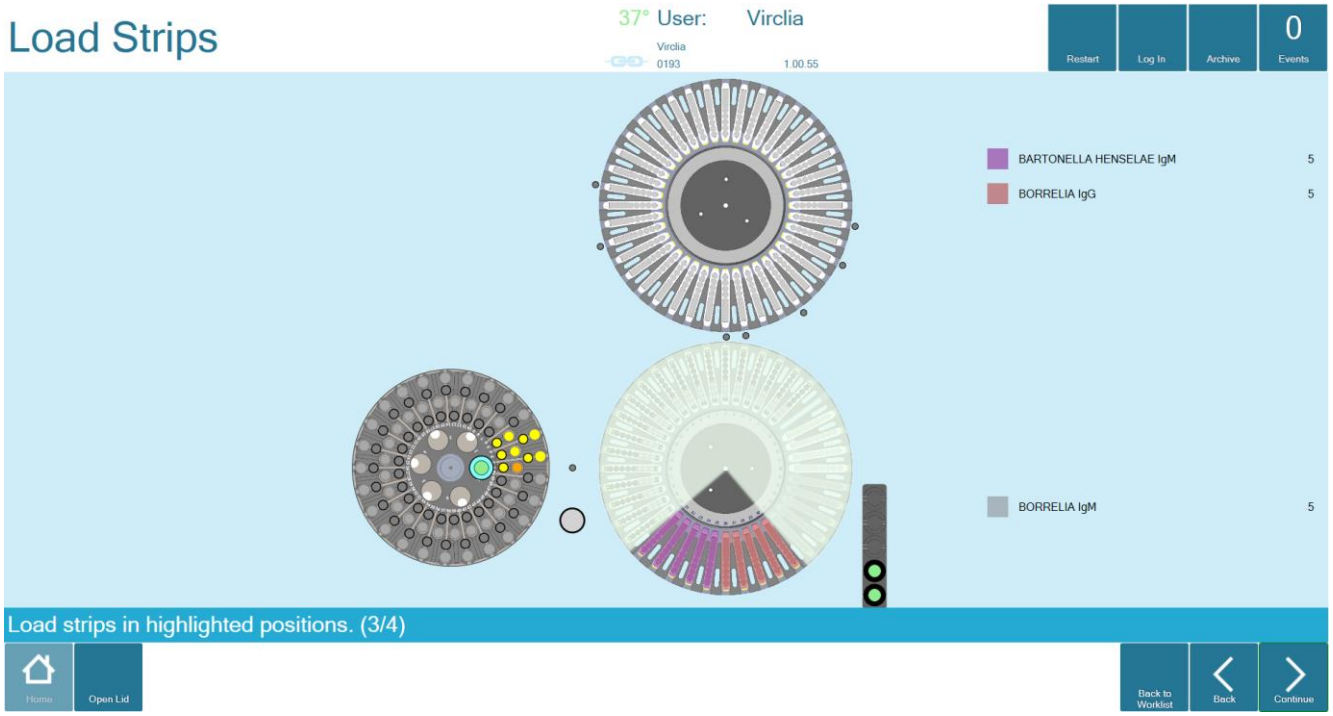
3.3.8 LOAD STRIPS SCREEN

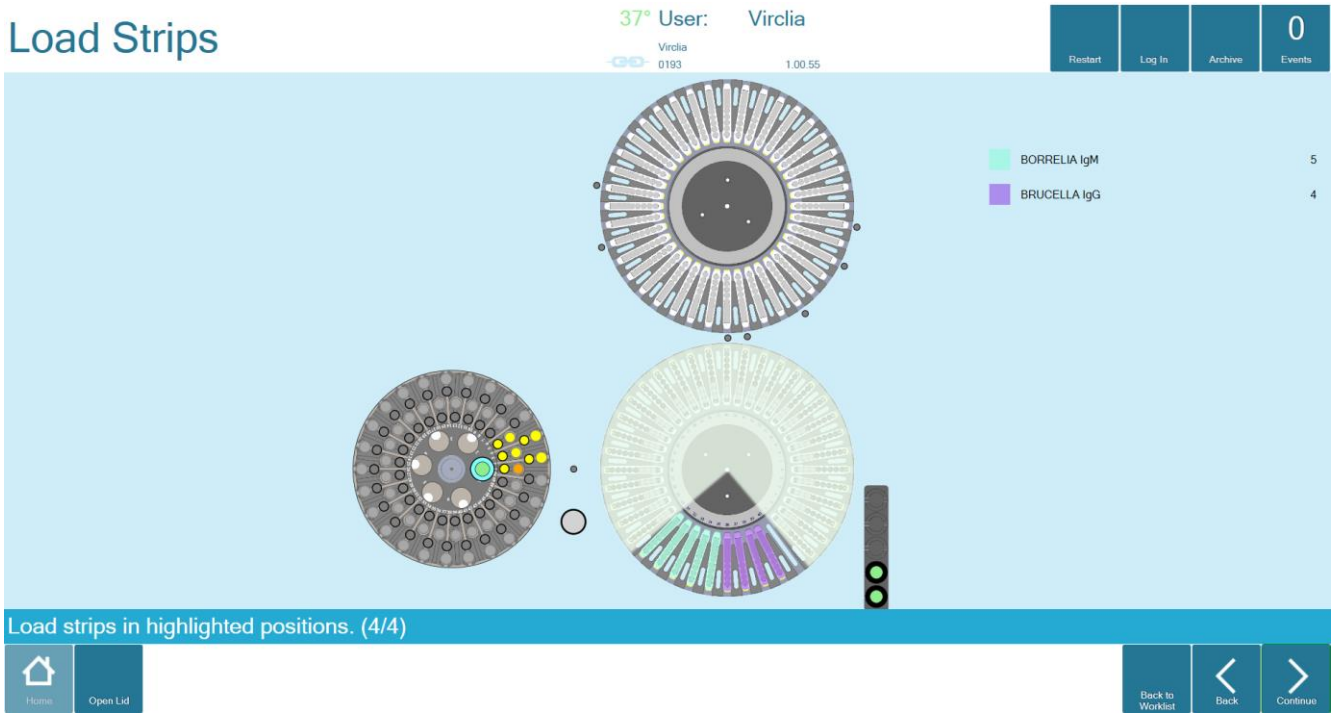
Through the "Load Strips" screens, the software guides the user to correctly load VirClia strips. Each type of VirClia strip is represented with a different colour. The colour assignment is shown in a table located on the right side of the screen. There can be up to 4 "Load Strips" screens depending on the number of VirClia Strips (1/4 to 4/4).
The instructions to load up to ten VirClia strips are shown on each screen. The colours assigned to some strip type in one screen may be repeated for another Strip type in another screen. The colour assignments are used to help the user distinguish between the different strip types within each individual screen.



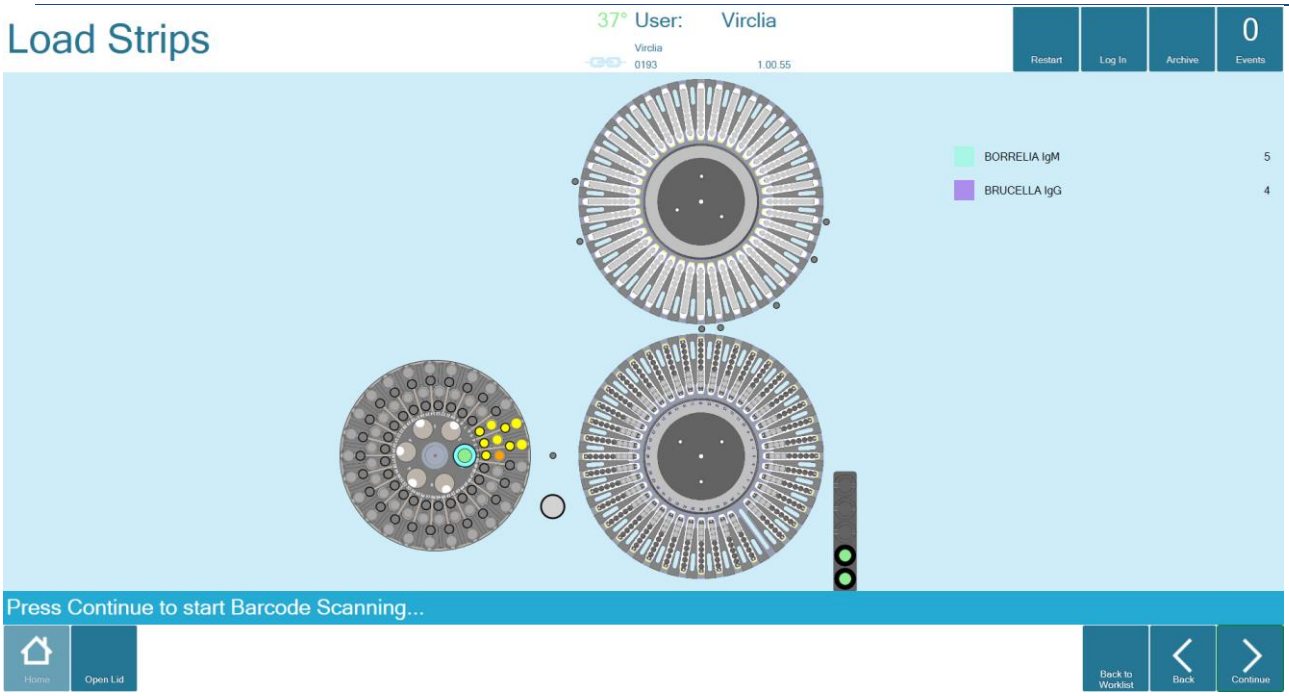
Press “Continue” to proceed to the next screen and the next 10 free positions will be shown. See below an example of four “Load Strips” screens:



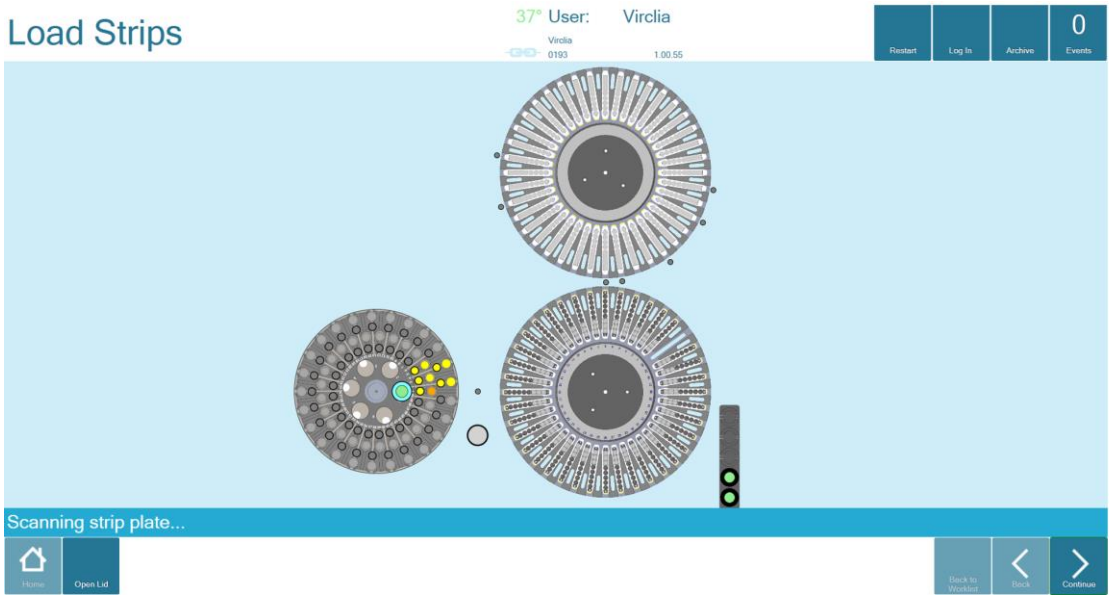




When all the strips are loaded, the software asks the user to click “Continue” to perform the barcode scanning:



The user is requested to close the lid to start the scanning. Before the run starts, the software verifies that all VirClia strips are correctly placed by reading the 2D barcode printed on them with the reader located over the rear side of the strip rotor.



The 2D barcode contains the lot number and expiry date of the VirClia strips. It is recommended to carefully manipulate the monotest strips avoiding cleaning them with alcohol and/or other reagents which could deteriorate the 2D barcode.

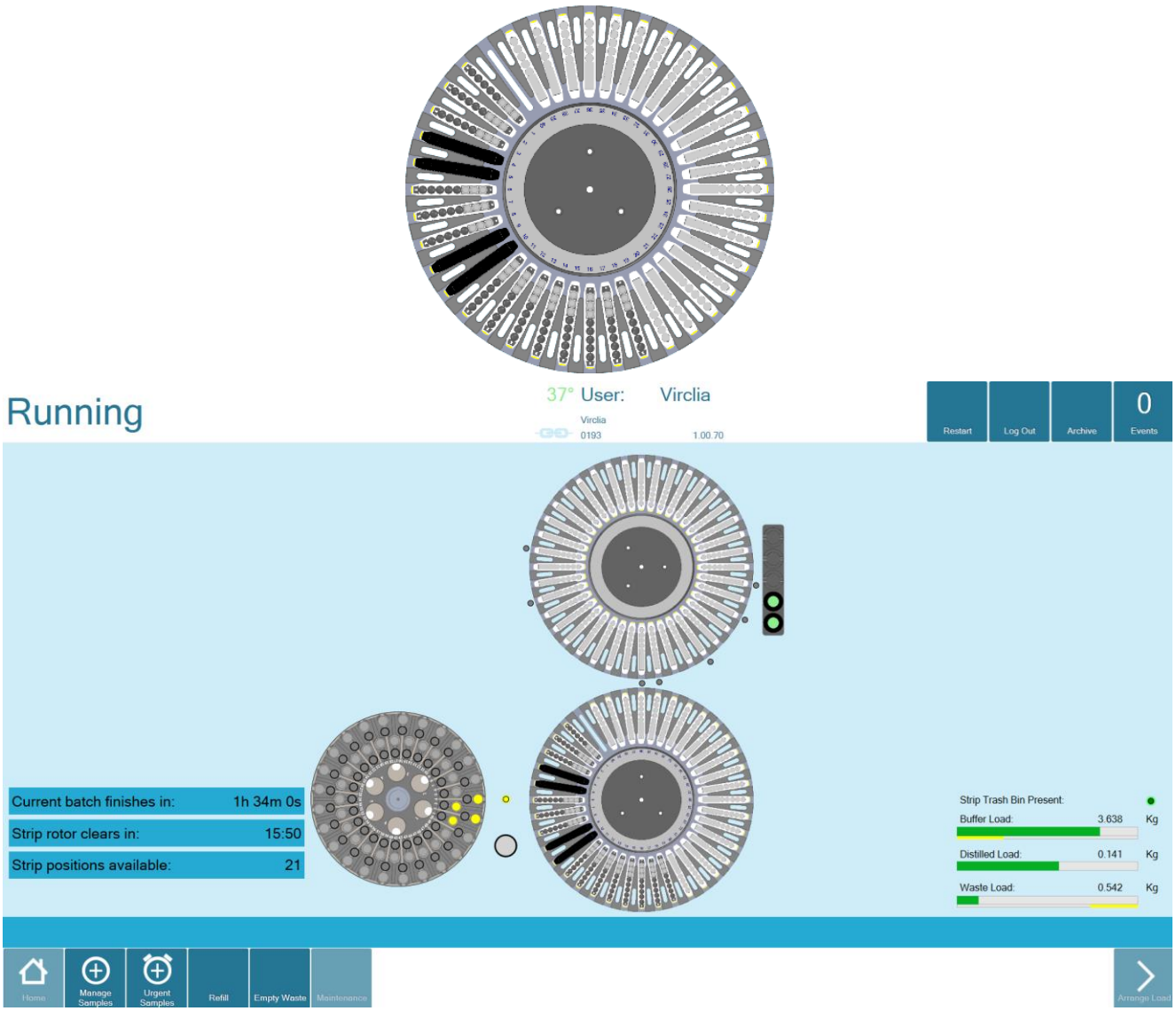
At the end of the scan cycle, if an error condition occurs (strip misplacement, unreadable 2D barcode or expired strip) the user is warned to replace the strip or to manually input the strip information:

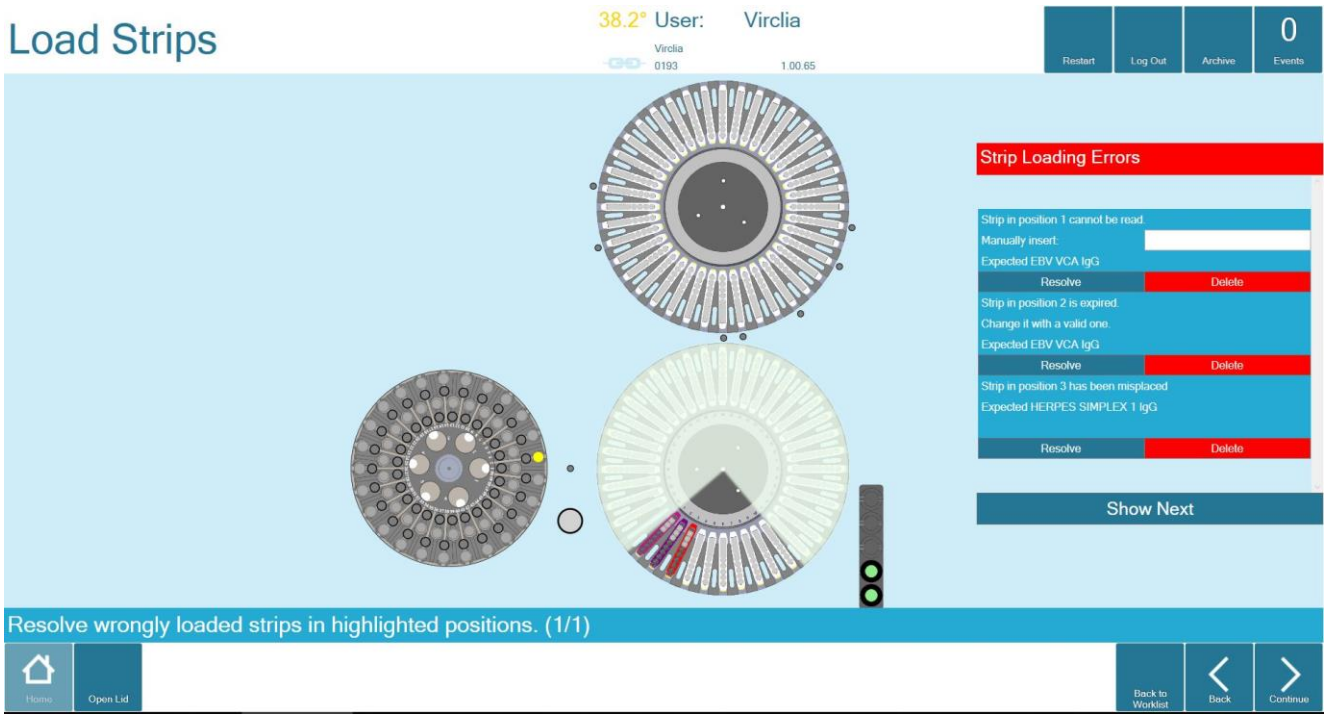
- **Wrong Strip:** the strip is highlighted in red in the strip rotor. Click on the “Resolve” button so the strip moves to the front and the user can replace it.

- **Unreadable 2D barcode:** the strip is highlighted in violet in the strip rotor. Insert the lot number manually and click the “Resolve” button.
- **Expired Strip:** the strip is highlighted in purple in the strip rotor. The software asks for a strip that is not expired. Replace the strip in order to proceed.

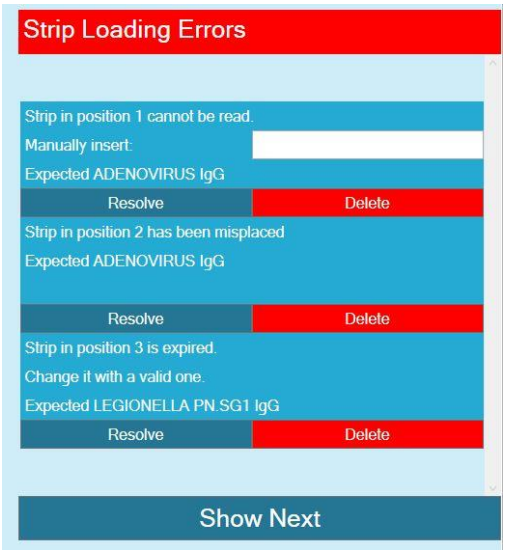
If the “Delete” button is double clicked, the strip will disappear from the screen. It will physically remain in the strip rotor. The deleted strip will not be analyzed, so the user does not have to remove it from the strip rotor at this step.
If the holder where the deleted strip was placed is required for further analysis (for example, during a continuous loading procedure), the software will ask the user to remove the deleted strip in order to free that holder position.

Note that if a certain strip is not detected by the optical sensor, the strip will be highlighted in black.

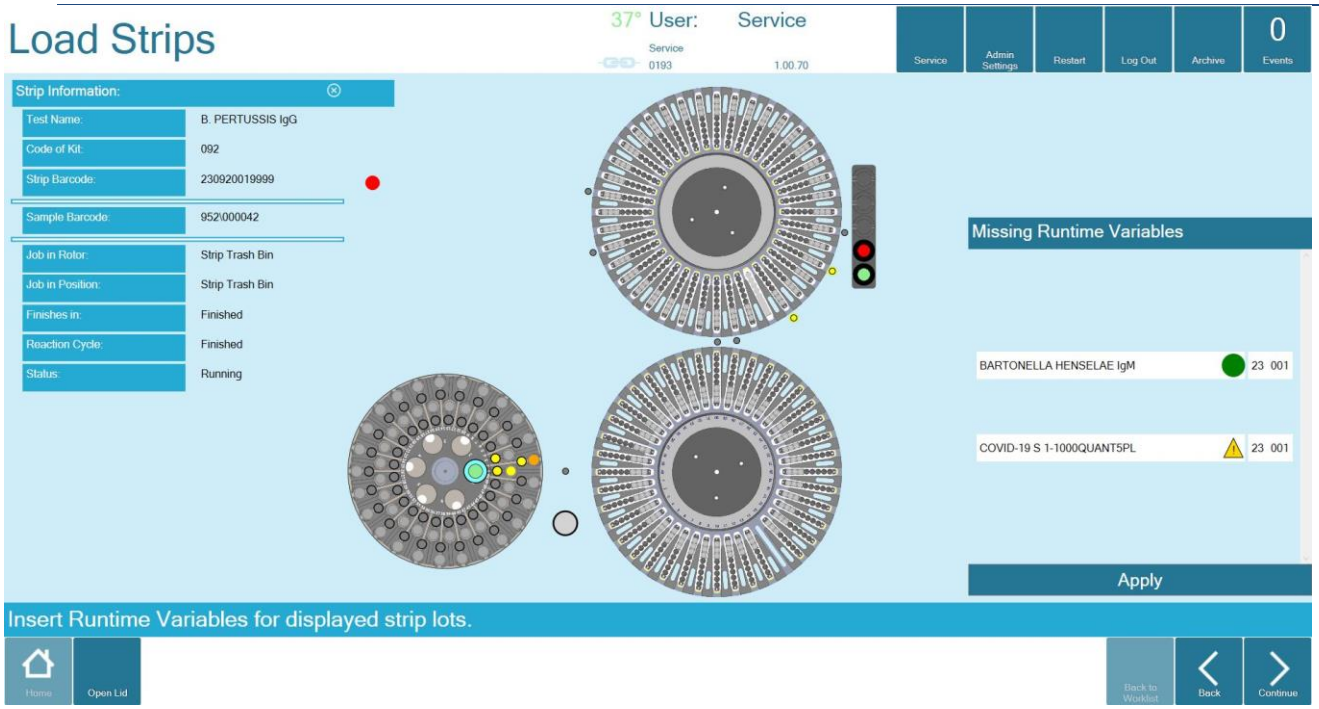




By clicking on the “Show Next” button, the Strip Rotor moves to show other strips with errors. After resolving all the error conditions, click “Continue”.

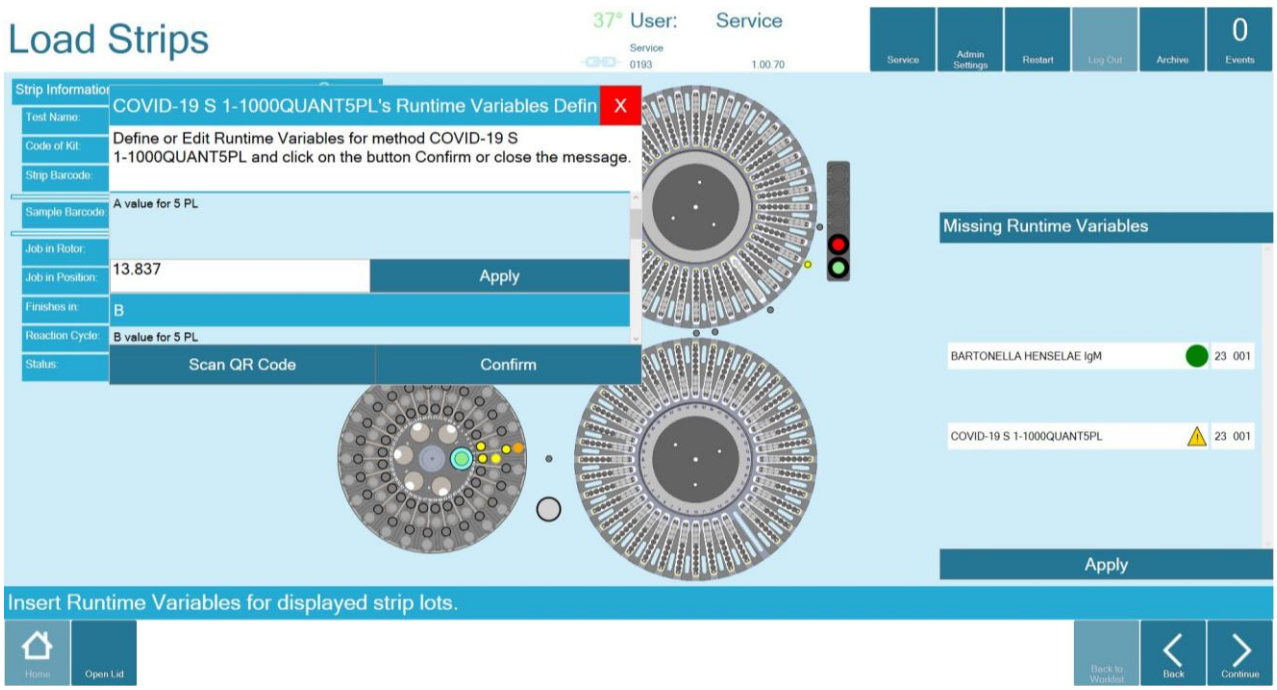


After the strip scanning procedure, the software will ask for typing the 5PL runtime variables for those methods which require them (and which have not already been typed in any previous assay). Click on the symbol ⚠ to type the requested runtime variables.



The software will display a pop-up window with the fields of the runtime variables that must be typed and the values stored by default. Type each value in the corresponding field, ensuring that the “Apply” button is clicked in each case and finally click on the “Confirm” button. Note that it is possible to populate all the fields by means of the “Scan QR code” if a handheld scanner is connected and configured.

This procedure is necessary for each new lot only the first time it is used in the instrument.



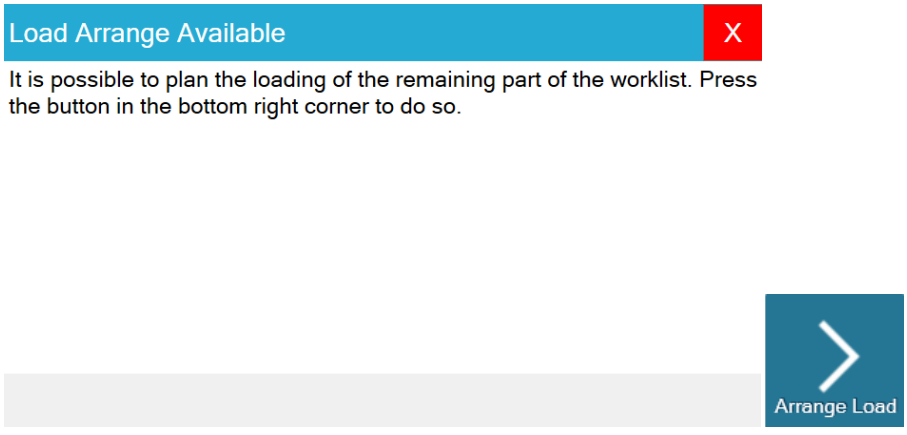
3.3.9 SAMPLES ANOMALIES

Before starting the run, the software performs a positive identification of the samples included in the sample rotor.
For those samples edited manually, VirClia Lotus™ requests the user to “Invalidate” or “Confirm” the assays assigned to them.
In the case any of the samples identified with barcode are not in their original position, the “Samples Anomalies” screen informs the user to place them back. Otherwise, the assays assigned to that sample will be invalidated.

Along this process, a new scan can be performed by clicking on “Scan”. In addition, the Sample Rotor can be accessed by clicking on “Open Lid”.

3.3.10 ARRANGE LOAD

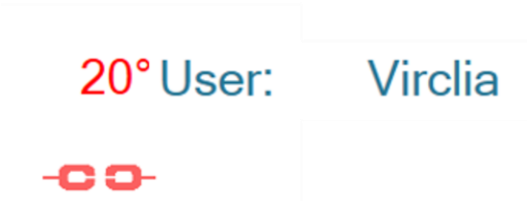
VirClia Lotus™ can operate in continuous mode. If the run contains more than 39 strips, the software will ask for an “Arrange Load” when new positions are available. This feature allows the user lo load new strips in order to save time while the instrument is processing the strips already loaded. In this way, the instrument efficiency is enhanced.



3.3.11 START THE ANALYSIS

The temperature is shown in yellow while it is not in the working range and in green when it reaches its optimal value. The run will start automatically when the optimal temperature is reached.

Note that if the temperature number is shown in red, it means that the instrument was not initialized yet:

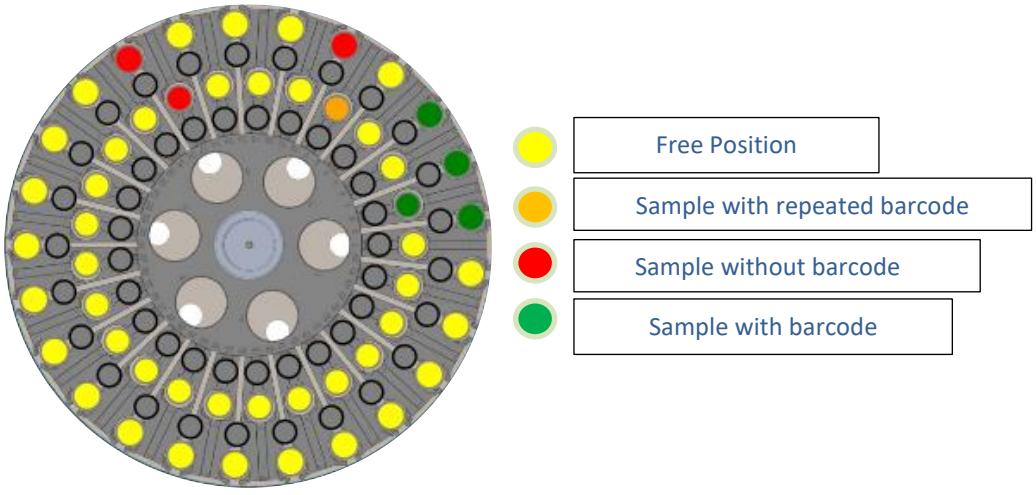


3.3.12 RUNNING SCREEN (part 2)

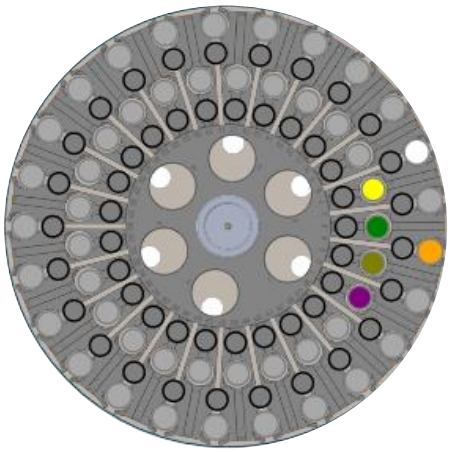
The “Running” screen appears again when the assay starts. It displays information related to samples, strips, predilution tubes and reagents.

The user will notice that the samples in the sample rotor have different colours, which will change as the assay proceeds. The meaning for every colour code is shown in the image below:

Before assigning tests to each sample:

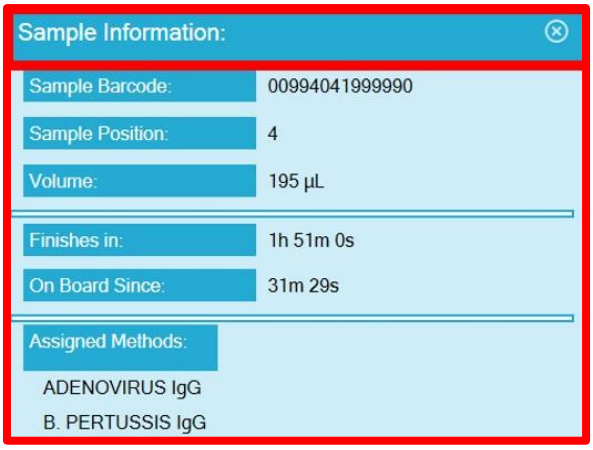


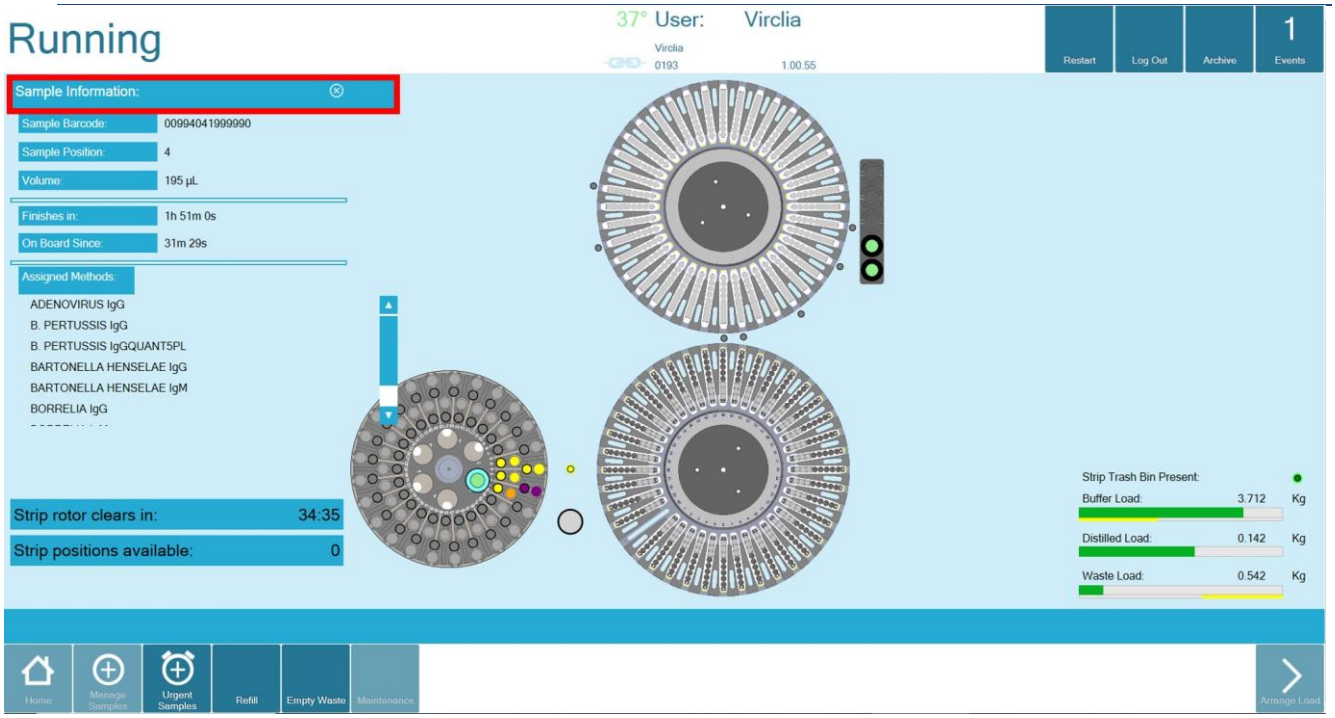
Once the tests have been assigned:



- Sample no identified or free position
- Sample without test assigned
- Scanned sample with test assigned
- Scanned sample and processed
- Sample manually identified with test assigned
- Sample manually identified and processed
- Aborted sample

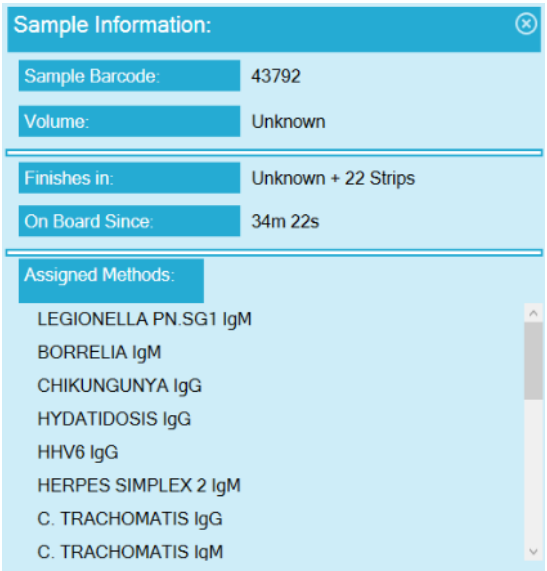
By clicking on the objects, all the details regarding that object are displayed on the left side of the screen. For example, if you click on a sample in the sample rotor, the information about this sample is shown on the left side.



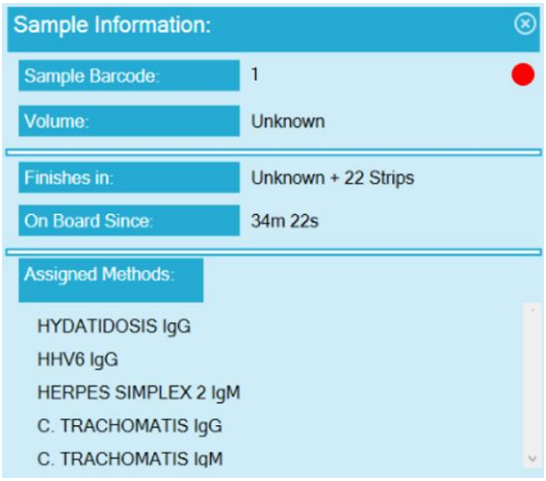


An example of the information displayed for a sample tube is shown in the figures below. If the sample has been manually identified, the software marks it with a red dot. Otherwise, no mark is shown.

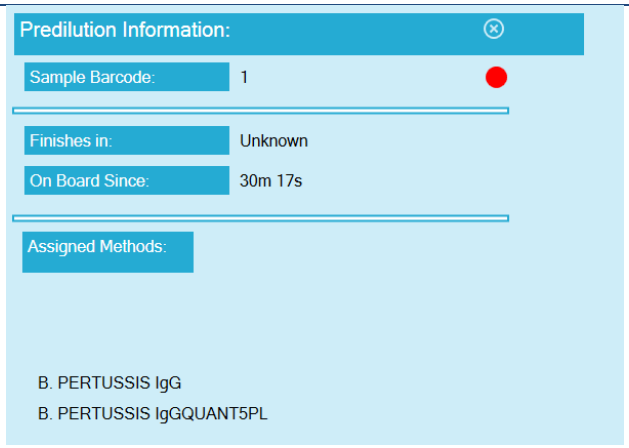
SAMPLE WITH BARCODE



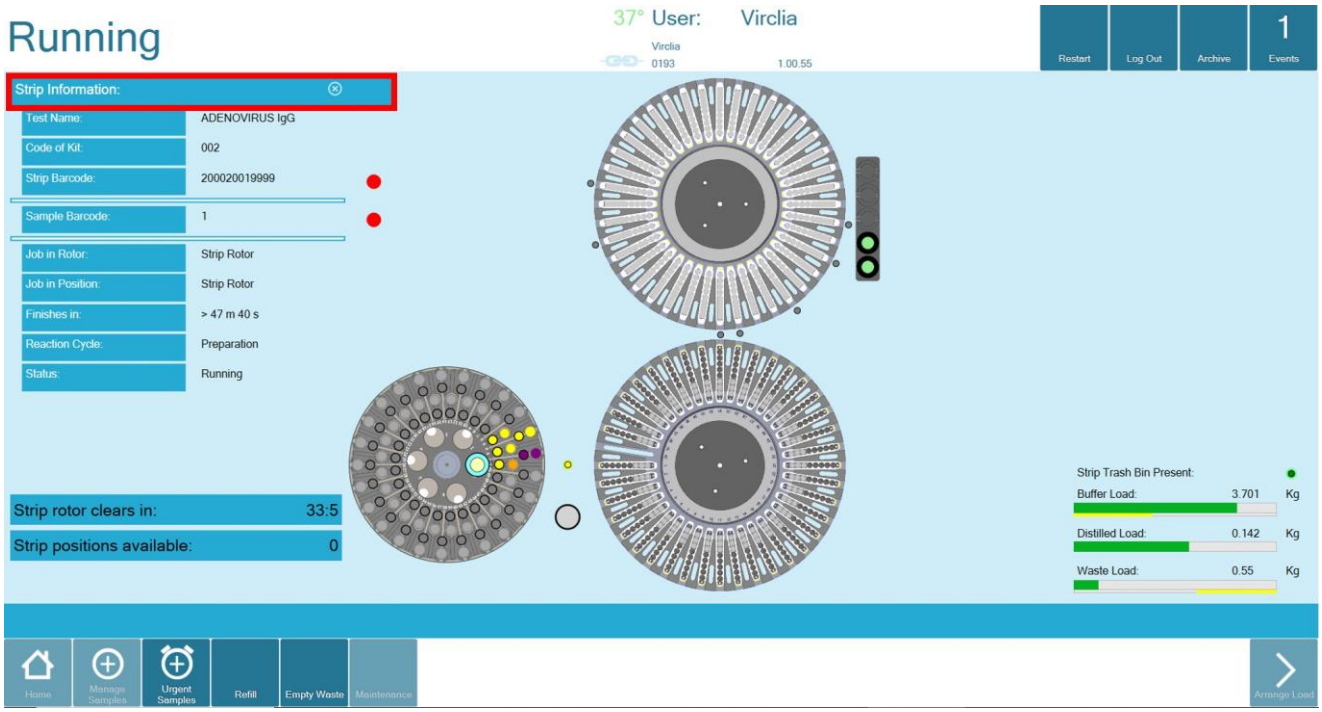
SAMPLE MANUALLY IDENTIFIED



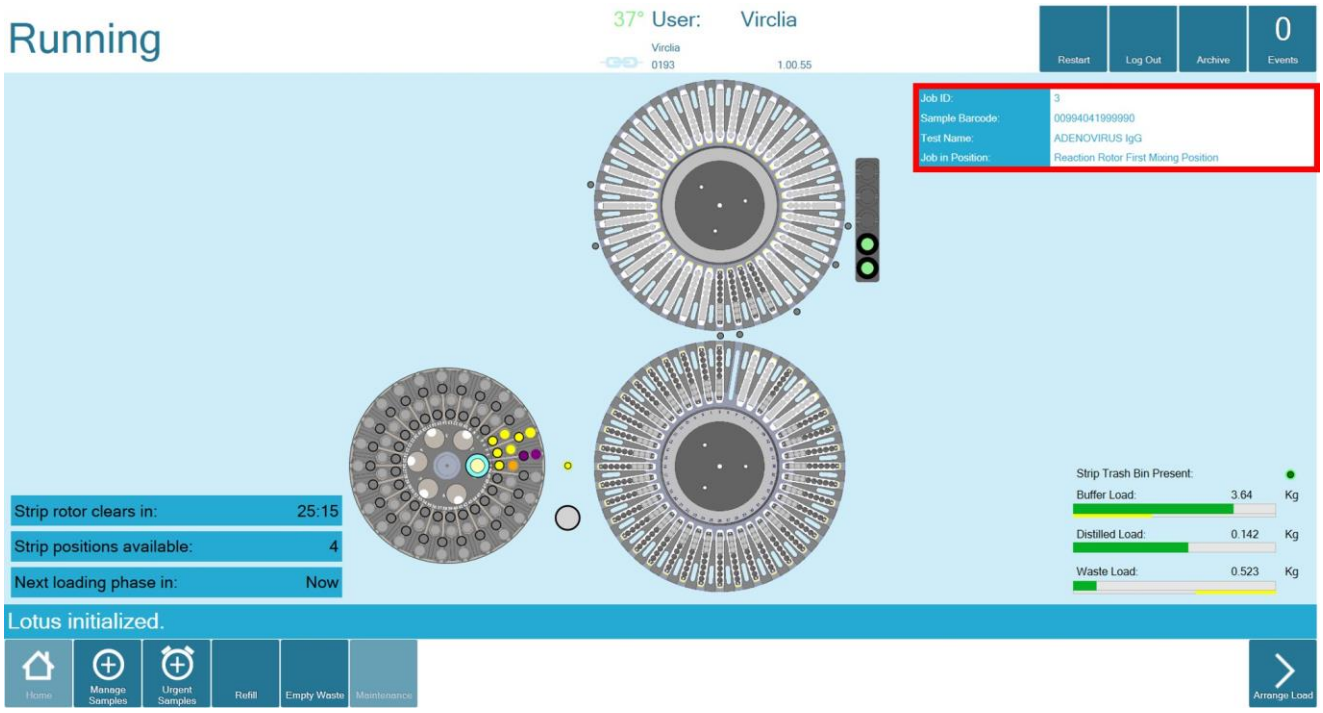
Predilution tubes information is always displayed with a red dot, as they are not barcoded.
If you click on a VirClia strip, either in the strip rotor or reaction rotor, the information about this strip is also shown on the left side.



By clicking one strip, no matter if it is inside the strip rotor or the reaction rotor, the information about the strip will appear in the left upper corner.

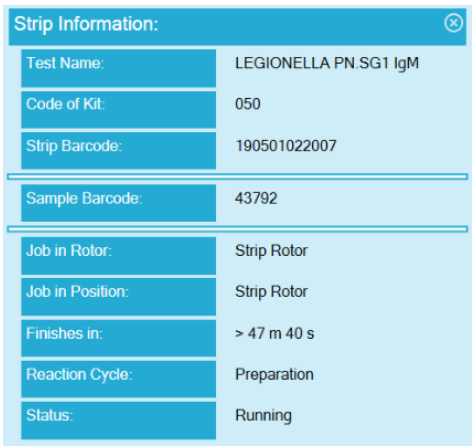


When a VirClia strip is in one of the reaction rotor stations (i.e. washer, reagent needle, reader) and this station is working, the information is automatically shown on the right side.

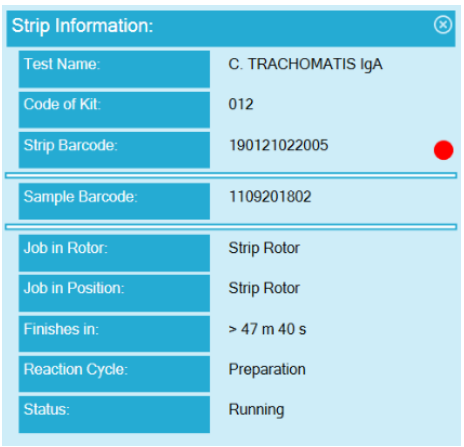


An example of the information displayed for strips is shown below. If the barcode on the strip has been automatically read, the number of the barcode is displayed unmarked. If the barcode of the strip is inserted manually, the software marks it with a red dot.

STRIP IDENTIFIED BY 2D BARCODE (STRIP ROTOR)

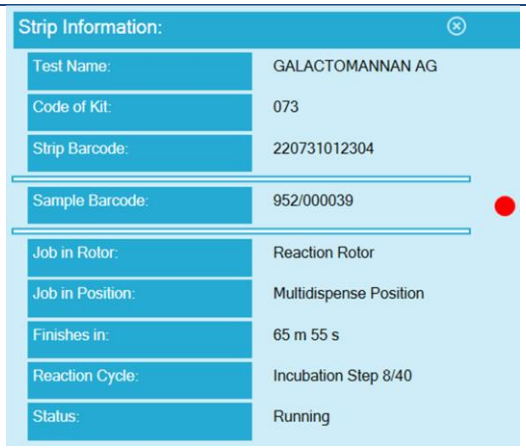


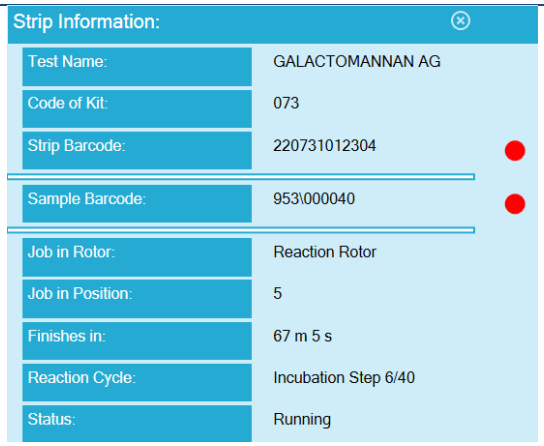
STRIP MANUALLY IDENTIFIED (STRIP ROTOR)



STRIP IDENTIFIED BY 2D BARCODE (REACTION ROTOR) MANUALLY TYPED SAMPLE ID

STRIP MANUALLY IDENTIFIED (REACTION ROTOR) MANUALLY TYPED SAMPLE ID





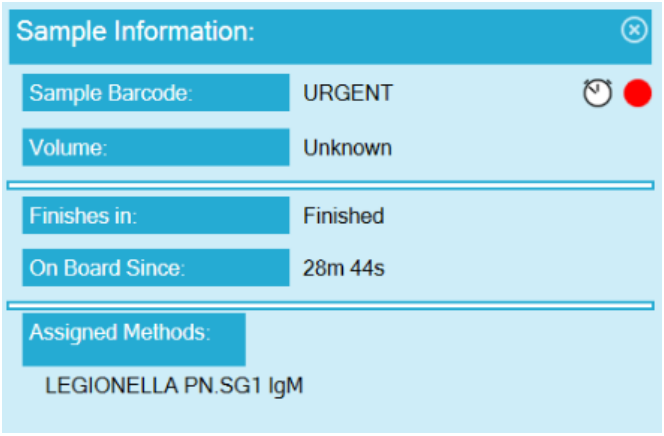
3.3.13 CONTINUOUS LOADING

During the run, it is possible to add new samples and strips, without stopping the assay. Click on the buttons “Manage Samples” or “Urgent Sample” to send the instrument a request to access the sample and the strip rotors.

The instrument shall complete any action that is being performed on the already loaded samples and strips, and then after the strips are transferred to the reaction rotor, the lid is released and new samples can be loaded. The user is informed that it is possible to open the lid to add new samples. The sample and the strip loading process is performed as described in previous sections. The samples inserted as urgent samples are highlighted in yellow.

Do not remove any already loaded strip while loading the new ones, as it could reduce the performance of the instrument.

All urgent sample strips will be processed with priority, while strips assigned to normal samples already present in the strip rotor will be processed after the urgent sample strips. A clock symbol identifies urgent samples.



3.3.14 RESOURCES LOADING DURING THE ASSAY

During the analysis, wash buffer tank and decontamination solution bottles can be refilled when required. In order to do that, Lotus Application offers three possibilities when the "Refill" button is clicked:

1) **Wash buffer refill:** This option allows the user to refill wash buffer only. If the instrument is using the wash buffer at the moment of clicking the refill button, the software will wait until the instrument is not using this solution. If not in use, the user will be able to take the wash buffer container out and refill it with fresh solution. After placing the container back, the instrument will prime the hydraulic circuit with the fresh wash buffer solution. The user will have 180 seconds to complete this action, otherwise some strips being analyzed could be affected.

   Care must be taken when the strips inside the reaction rotor are nearby the strips washing station. The user can check which strips could get compromised if a refill of wash buffer is performed simply by placing the cursor over the refill button. It is at the user's risk to perform the refill if the strips are too close to the strips washing station.

2) **Decontamination solution refill:** This option allows the user to refill decontamination solution only. If the instrument is using the decontamination solution at the moment of clicking the refill button, the software will wait until the instrument is not using this solution. If not in use, the instrument will place the auxiliary reagent rack in the operator's position and the user will be able to refill (or alternatively replace) either one or both of the decontaminant solution bottles. Likewise, the user will have 180 seconds to complete this action.

   Care must be taken when the strips inside the reaction rotor are nearby the reagent needle station. It is at the user's risk to perform the refill if the strips are too close to the reagent needle station.

3) **Wash buffer and decontamination solution refill:** This option allows the user to perform the refill of both wash buffer and decontamination solution. The already mentioned considerations for the refill of the individual resources must be taken into account at this step.

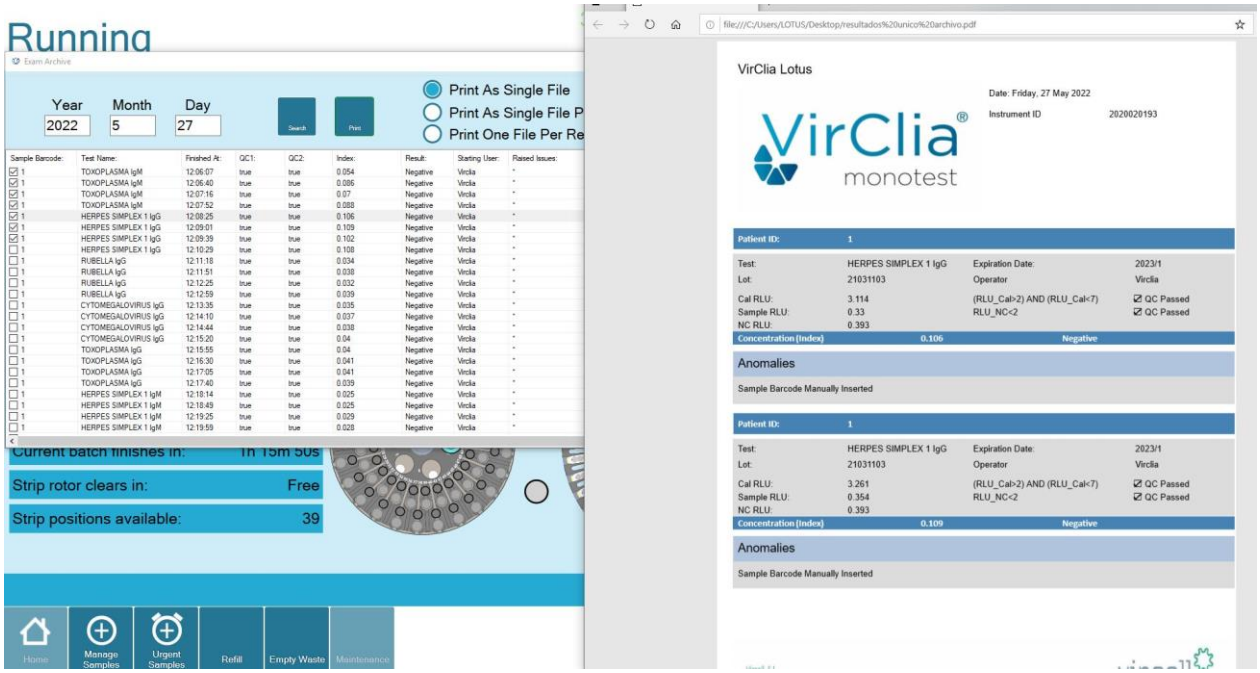
3.3.15 RESULTS

After completion of the analysis process, the result is calculated and saved in a file located in the program folder. If the LIS connection is active, it will be possible to transfer results to the laboratory host computer. Results can be preliminary reviewed by clicking on the "Archive" tab. For an easier handling of results information, it is recommended to use the VirCom middleware.

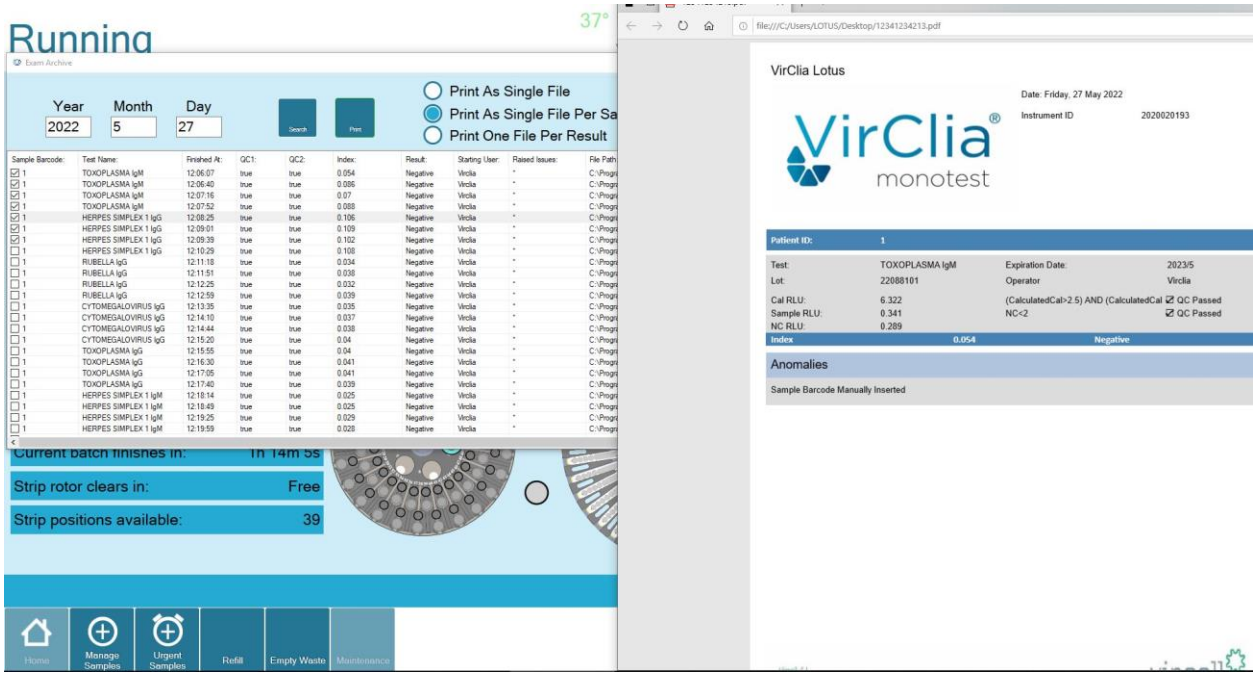
In the "Archive" tab, type the desired year, month and day, and press search to show results for the selected date.

The user can generate reports in the three modes:

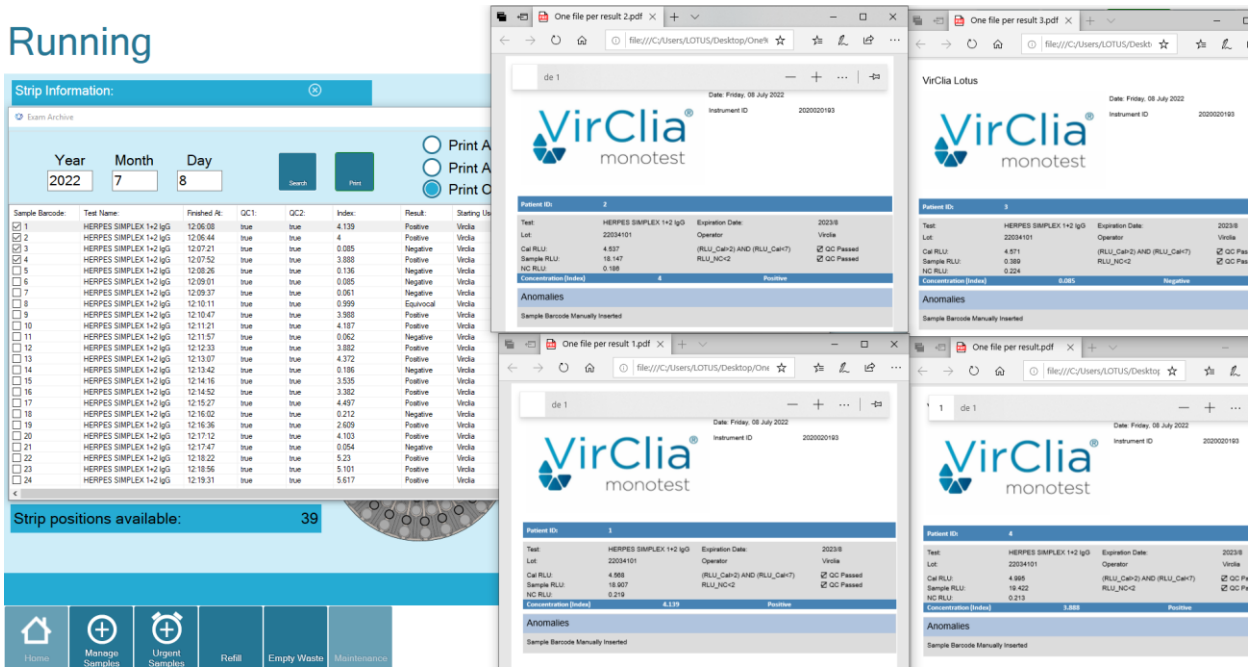
1. **As a single file**. All the results are merged in a single PDF document.



2. **As a single file per sample**. All the results obtained for each sample are generated in individual PDF files.



3. **Print one file per result**. Each result is generated in an individual PDF file.



Click again on the “Search” button to refresh the screen.

In a report file, the user can find the following information:


VirClia Lotus

VirClia® monotest

Date: Friday, 27 May 2022

Instrument ID 2020020193

Patient ID:	1	1			
Test:	2	HERPES SIMPLEX 1 IgG	Expiration Date:	5	2023/1
Lot:	3	21031103	Operator	6	Virclia
Cal RLU:	4	3.114	(RLU_Cal>2) AND (RLU_Cal<7)	7	☑ QC Passed
Sample RLU:		0.33	RLU_NC<2		☑ QC Passed
NC RLU:		0.393			
Concentration (Index)	8	0.106	9		Negative

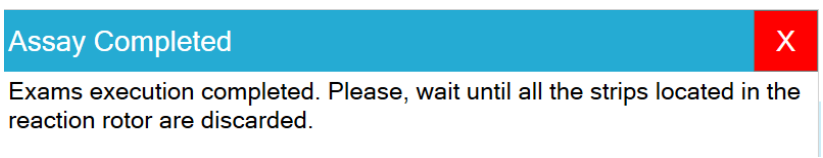
Anomalies 10

Sample Barcode Manually Inserted

1. Sample name
2. Test name
3. Strip lot number
4. RLU results of the calibrator, sample and negative control
5. Expiry date of the strip
6. Operator who performed the assay
7. Validation rules: A tick indicates that the QC criteria passed
8. Result obtained (in qualitative tests, it will be an index value. In quantitative tests, it will be a concentration value)
9. Interpretation
10. Anomalies that occurred during testing (if any).

3.3.16 END OF WORK

When all the analysis sessions have been completed, a pop-up message will appear in the screen:



Once finished, the user must perform a maintenance procedure before shutting down the instrument (refer to section 5.4. Daily Maintenance Procedure).

Chapter 4

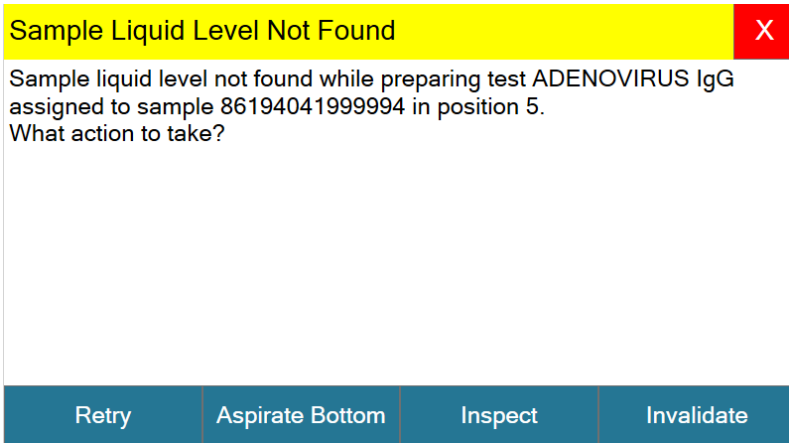
4. ERRORS & WARNING MESSAGES

During the run, the instrument software is continuously monitoring all the activities performed by the various motor actuators and pumps, using specific sensors.
In case some warning or error condition is detected, a message box is displayed and the user is requested to perform a corrective action to manage the situation. In addition, the frontal LED will blink showing different colors depending on the situation of the instrument, as shown below:

- **Blue (blinking):** normal status.
- **Yellow, Red (blinking):** user interaction required; pop-up window is open on the application.
- **Red (blinking):** PC used to interact with the instrument and has not requested anything for more than one minute.
- **Red, Green, Blue (blinking):** powering on / clear before start.
- **Green (blinking):** running exams have been completed.

4.1 FAILURE IN SAMPLE OR REAGENT LIQUID LEVEL DETECTION

The Sample and Reagent needles are able to detect the liquid inside the sample tubes or the reagent bottles.
If the Sample needle does not detect liquid in a sample tube, the following message is displayed:

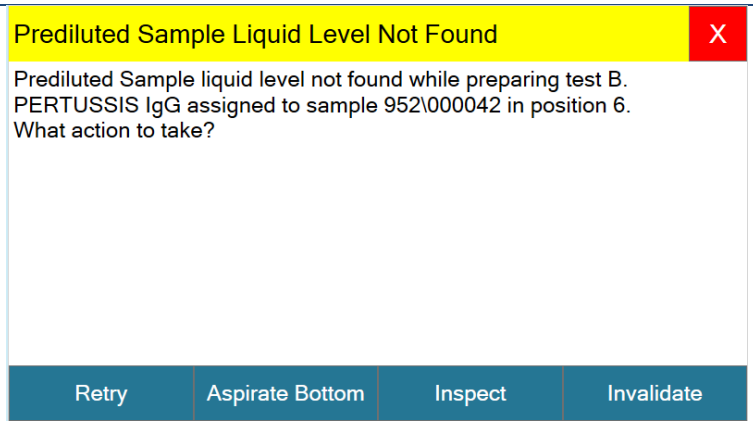


Interpretation:
The instrument could not detect liquid inside the sample tube.

Root causes:

- Sample needle is misaligned.
- Sample volume is low.

If the sample needle does not detect liquid in a predilution tube, the following message will be displayed:



Interpretation:

The instrument could not detect liquid inside the predilution tube.

Root causes:

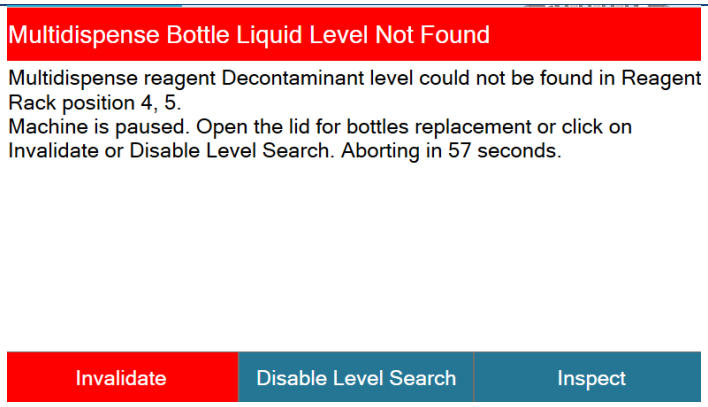
- Sample needle is misaligned.
- Prediluted sample volume is low.

The user is requested to perform one of the following actions:

Retry	Repeat the automatic level detection.
Aspirate from the Bottom	Force the instrument to aspirate the liquid from the bottom of the container. Press this option only if you are sure there is enough liquid inside the tube or bottle.
Inspect	The cover is unlocked to allow the user to open it in order to inspect the container and refill it. Press the **Retry** command after the refill.  - Pay attention to avoid mixing liquids during the refill operation.
Invalidate	Skip the specific container and invalidate the programmed assays assigned to that sample/reagent.

After a configurable wait time, this screen will shut down and the “aspirate from the bottom” will be performed automatically.

If the reagent needle does not detect liquid in one of the reagent bottles, the following message is displayed:



Interpretation:

The instrument could not detect liquid inside a bottle of decontaminant solution or dissociating solution.

Root causes:

- Reagent needle is misaligned.
- Decontaminant solution bottle volume is low.
- Dissociating solution bottle volume is low.

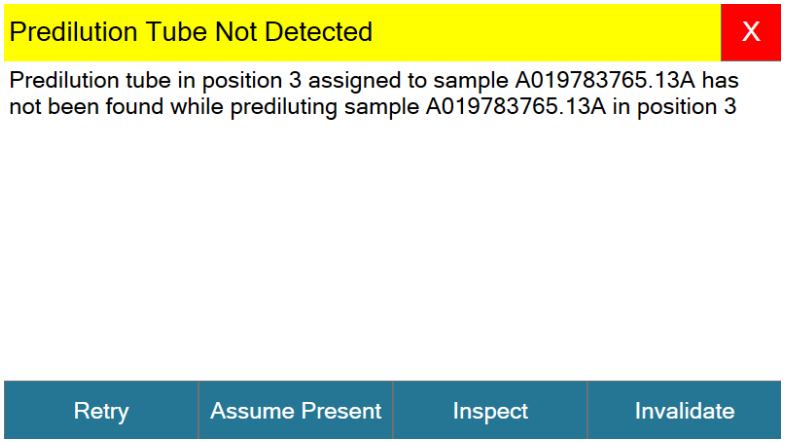
The user is requested to perform one of the following actions:

Disable Level Search	The needle aspirates from the bottom of the reagent bottle.
Invalidate	Invalidate the programmed assay assigned to that reagent.
Inspect	The cover is unlocked to allow the user to open it in order to inspect the container and refill it. Press the **Retry** command after the refill. ⚠ - Pay attention to avoid mixing liquids during the refill operation.

After a configurable wait time, this screen will shut down and the probe will aspirate from the bottom of the reagent bottle.

4.2 PREDILUTION TUBE DETECTION

An alert message is displayed informing the user that a Predilution tube has not been detected in the right position:



Interpretation:

The instrument could not detect the presence of a certain predilution tube.

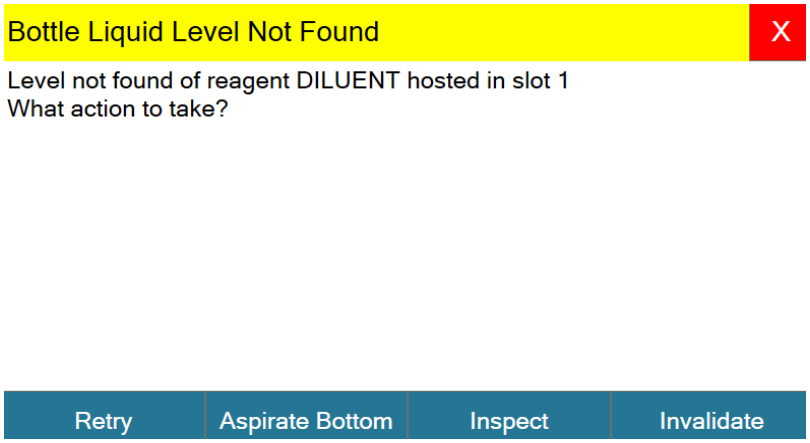
Root causes:

- Predilution tube sensor is not correctly calibrated or it is faulty.
- Predilution tube is not placed in the indicated position.

The user is requested to perform one of the following actions:

Retry	Repeat the automatic tube detection.
Assume Presence	Force the instrument to assume that the predilution tube is present inside the sample rotor. Make sure that the tube is present to avoid any liquid spillage
Inspect	The cover is unlocked to allow the user to open it in order to inspect the Predilution tube and replace it. Press the **Retry** command after the replacement.
Invalidate	Skip the Predilution tube and invalidate the programmed assays assigned to it.

4.3 BOTTLE LIQUID LEVEL NOT FOUND



Interpretation:

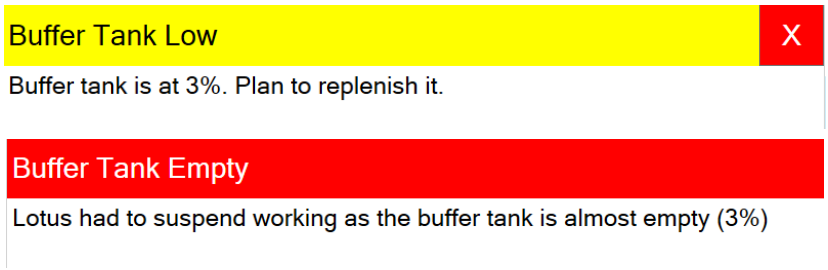
The instrument cannot detect liquid inside the dilution bottle.

Causes:

- The diluent bottle is not positioned in its correct location or it is empty.
- The sample needle is not well calibrated.

4.4 WASH BUFFER LOW VOLUME

The volume of the Wash Buffer tank is continuously monitored. If there is a low volume of wash buffer solution, this warning message will be displayed:



This situation can be cause by these situations:

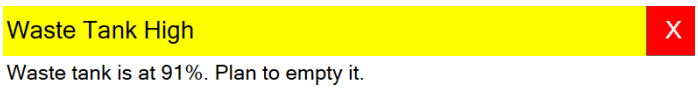
- There is no wash buffer solution in the wash buffer tank.
- The wash buffer weight scale is not well calibrated.
- Wash buffer weight scale or its associated circuit is faulty.

To solve the first situation, the user has to request a refill of wash buffer through the corresponding button:

Once more wash buffer solution has been placed in the wash buffer tank, the instrument will prime the hydraulic circuit with fresh wash buffer.

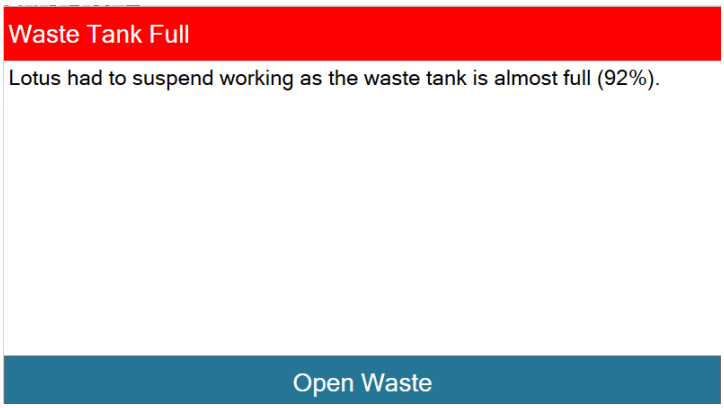
4.5 HIGH LEVEL WASTE TANK

The Waste Tank volume is continuously monitored. When the liquid waste content exceeds 91% of the total volume, a warning message is displayed:



The user will have to empty the contents of the waste tank using the corresponding button:

When the waste tank is full, the instrument stops working and the following warning message is shown:

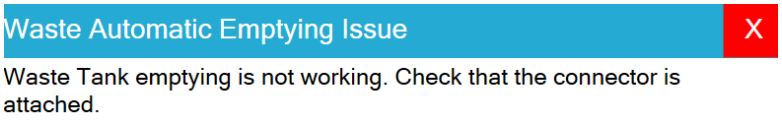


The user is requested to perform the following action:

Open waste	Press “Open waste” to empty the waste tank. **Some of the tests could be aborted in case the waste container is full!**

Vircell S.L. recommends to work with the auto empty waste function enabled (consult Technical Service for the correct configuration of this function) to avoid the manual emptying of the waste tank. In this way, the internal waste tank constantly drains its content to the external waste tank. If this mode is used and a warning appears, verify that the connection between the internal and the external tank is correct, and that there are not folds of the tube.

4.6 WASTE AUTOMATIC EMPTYING ISSUE



Interpretation:

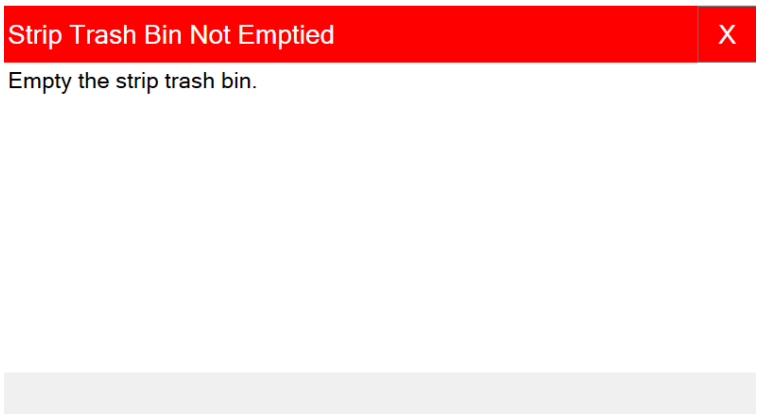
The automatic waste emptying cannot be performed.

Causes:

- The fast connector, which connects the external waste tank with the instrument, is not well placed.
- The fast connector is faulty.
- The valve of the fast connector is clogged.

4.7 STRIP WASTE CONTAINER FULL

The residual capacity of the Strip Waste Container is calculated based on the number of strips processed/disposed. When the number of discarded strips exceeds the maximum capacity (n=60 strips), the system stops discarding the strips, remaining on the Reaction Rotor until the container is emptied. A warning message is displayed as follows:



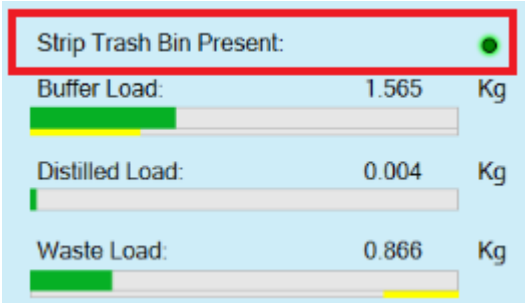
Close the warning, empty the Strip Waste Container and click “Continue” to proceed.

In the bottom right corner of the screen, the status of the Strip Waste Container is shown:

- Green dot: Container is present.
- Red dot: Container is absent.

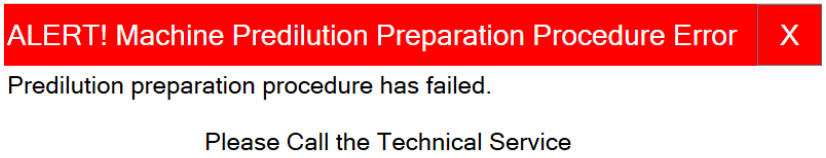
  When the container is absent, this pop up will appear until it is placed back in the correct position:

  If the dot remains in red even though the strip trash bin has been placed in the right position, the strip trash bin detector may be faulty. Contact the service engineer for checking this matter.
- Yellow dot: Container is almost full.

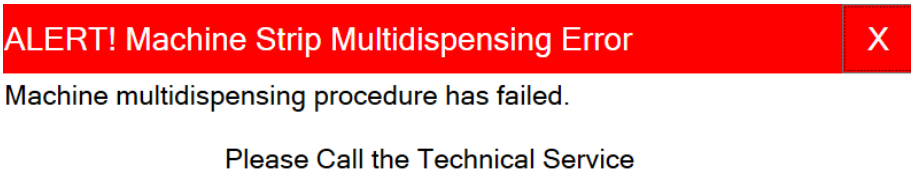


4.8 MOTOR OR HARDWARE ERROR

The instrument software is continuously monitoring all the motors and hydraulics activity. In case of error, an alert window is displayed and the user is requested call the Technical Service:



4.9 ALERT! MACHINE STRIP MULTIDISPENSING ERROR



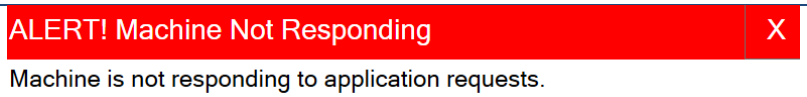
Interpretation:

The reagent needle has crashed against some obstacle during its movement.

Causes:

- The decontaminant bottle has the cap on.
- The reagent needle is not well calibrated.
- There is an obstacle that may interfere with its correct movement.

4.10 ALERT! MACHINE NOT RESPONDING



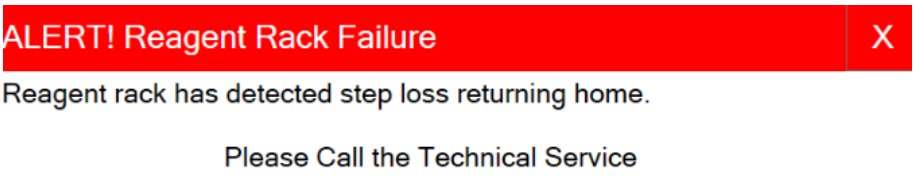
Interpretation:

The software cannot establish a connection with the instrument.

Causes:

- The USB cable between the instrument and the laptop is not connected or it is faulty.
- The instrument is switched off.

4.11 ALERT! REAGENT RACK FAILURE



Interpretation:

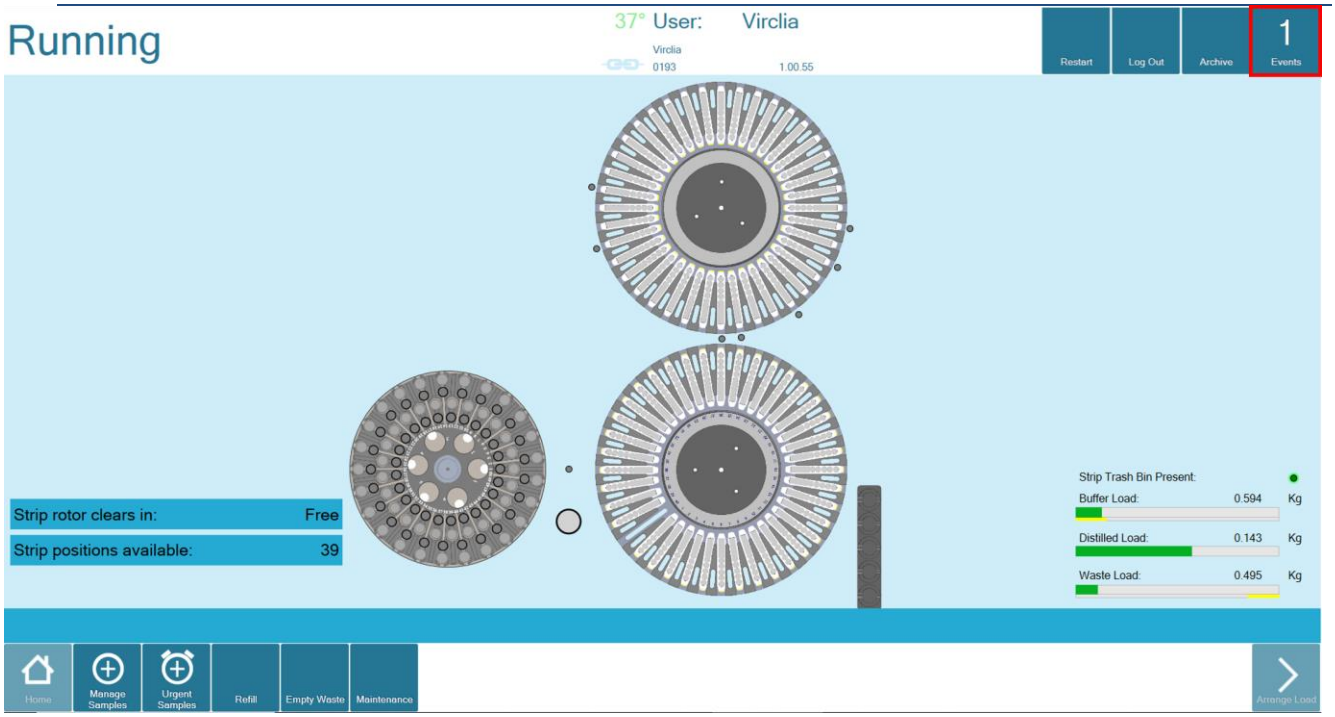
A step lost has been detected in the reagent rack.

Causes:

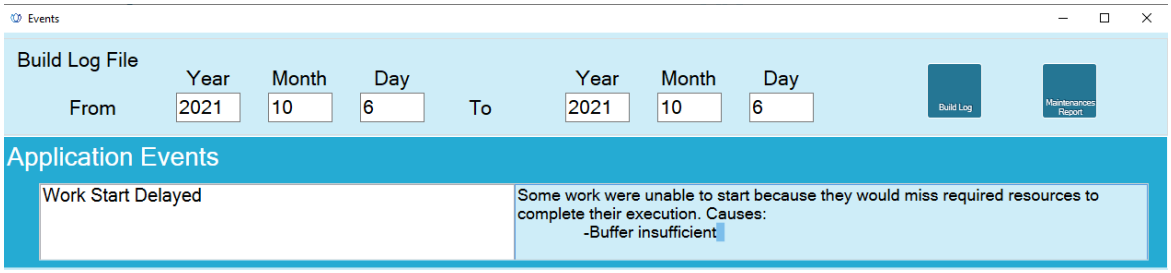
- The operator has interfered with the rack movement.
- There is an obstacle in the reagent rack which interferes with its correct movement.

4.12 EVENTS TAB

The majority of the already mentioned alerts can be visually checked in the main screen when the pop up appears and also in the events tab.



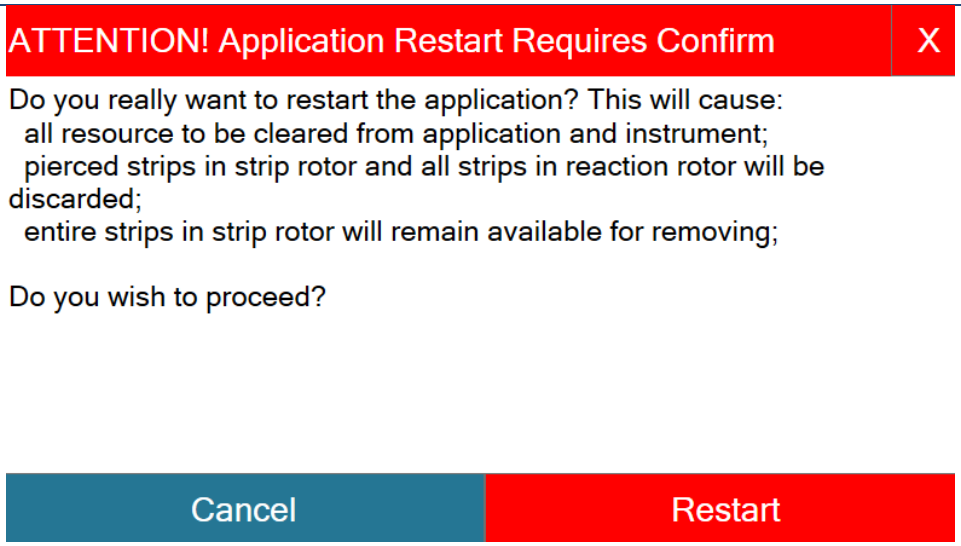
By clicking the Events button, the tab will be displayed. If any alert occurred during the run, a detailed information will appear in this tab:



4.13 RESTART

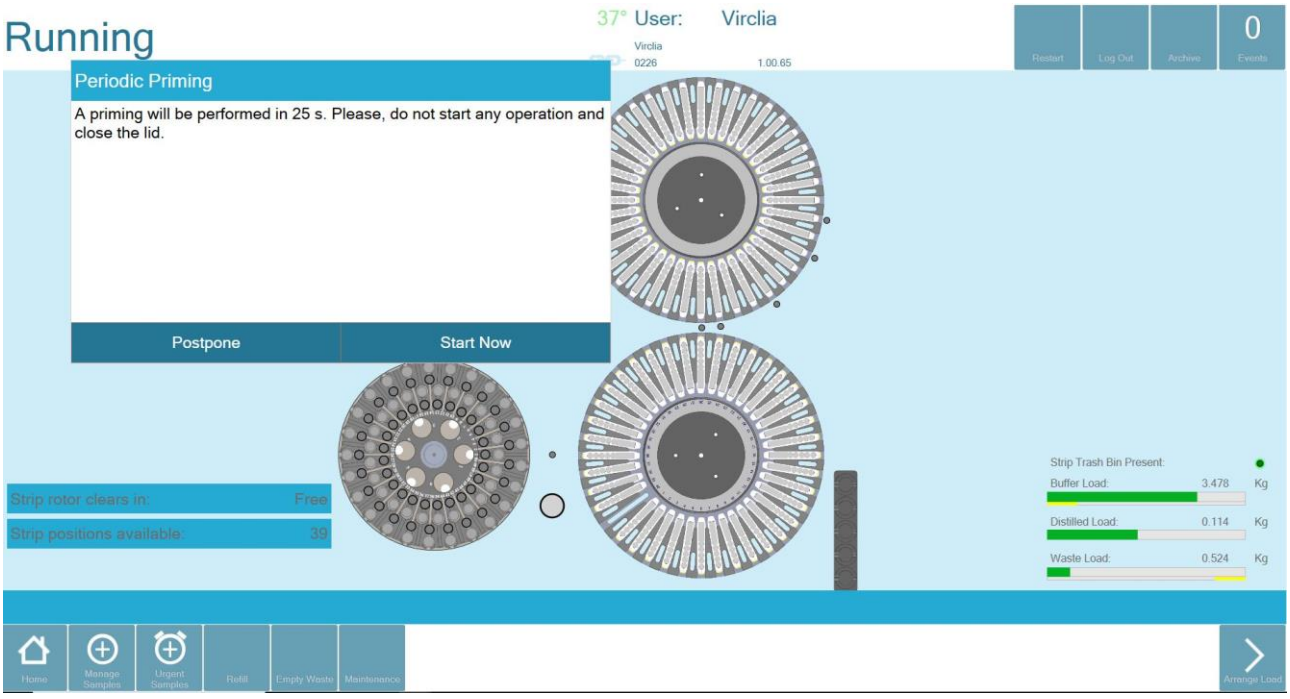
If the instrument gets frozen during the execution of an assay, it is possible to restart it by clicking on the tab “Restart”. By doing this, the following procedure will be carried out:

- All the moving parts will return to their home positions.
- Reaction rotor occupied positions are emptied.
- Strip rotor and reaction rotor checks the presence and the positions of all the strip holders and finds the free position.



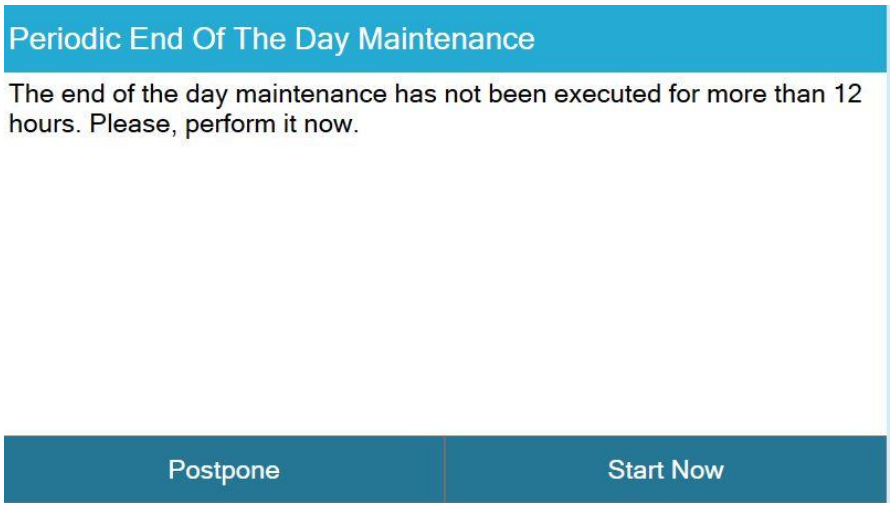
4.14 PERIODIC PRIMING

The time the instrument is idle is continuously monitored. When it exceeds 30 minutes, an automatic priming with wash buffer solution will be performed. Those priming cycles will be performed each 30 minutes continuously. If the user is performing any action in the instrument, the priming can be postponed or performed immediately.



4.15 PERIODIC END OF THE DAY MAINTENANCE

Once the instrument has been initialized, the time that happened since the last end of the day maintenance is continuously monitored. If the user decides to postpone the message, it will appear continuously every hour.



Chapter 5

5. MAINTENANCE

5.1 GENERAL RULES

WARNING: Improper maintenance of the VirClia Lotus™, may cause damage to the instrument.

Before performing the Instrument Maintenance Procedures, make sure that:

- The working area is clean and free of any remaining resources from the previous session.
- Possible spills in the working area have been immediately cleaned with water and left dry.

A continuous inspection of VirClia Lotus™ is necessary to ensure that the system works properly.

All materials used during a session, must be disposed of in accordance with local safety regulations.

In case of malfunction of the system, or breakage of any of the parts of the instrument, the repair or replacement must be carried out by by qualified and trained Technical Service.

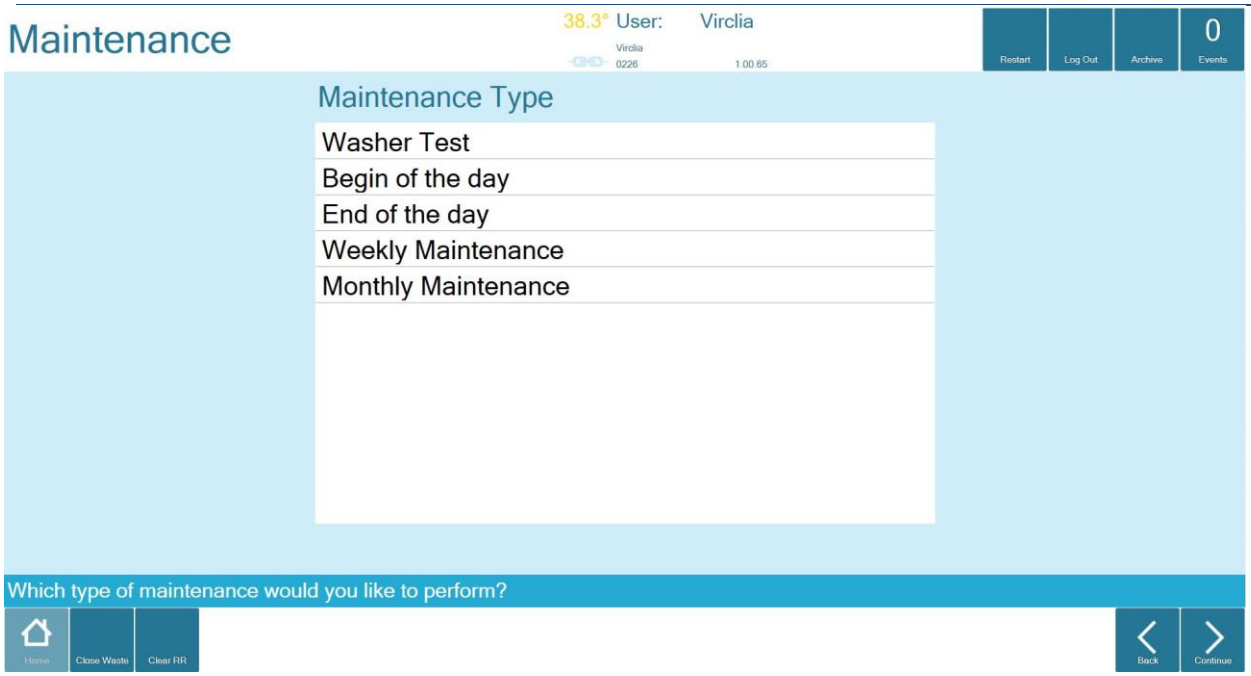
5.2 RESPONSIBILITIES

In case of breakdown of the system or of any of the parts, the Technical Service should be informed immediately. Daily and weekly maintenance procedures must be performed by an operator. Long-term maintenance will also be performed by the Technical Service.

5.3 INTRODUCTION

To access the different types of maintenance, click on the “Maintenance” tab located in the bottom left side of the main screen.

In this way, a new window appears where you can select each of different types of Maintenance: begin of the day, end of the day, weekly and monthly.



In the following sections, each type of maintenance is explained in detail.

5.4 DAILY MAINTENANCE PROCEDURE

Daily maintenance includes start-up and shutdown procedures for the instrument. The start-up steps are performed prior to the first run of the day. The shutdown steps are performed after the last run of the day.

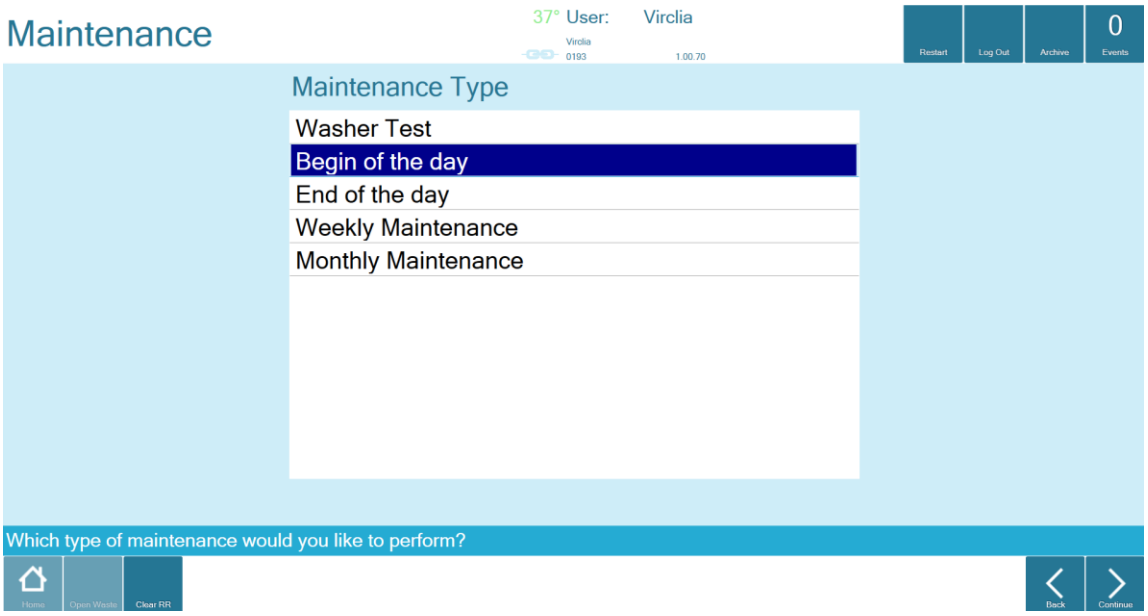
Begin of the day: This maintenance must be carried out before the first test of the day, in order to remove the distilled water inside the tubing system. This maintenance can be carried out in 2 ways:

1) Normally, this maintenance is performed when running the software, after having logged in with a user, using one of the following options:

   A) **Start Working**: this option should be chosen regularly by the end user. By clicking on this button, the hydraulic circuit of the instrument will be primed with wash buffer.

   B) **Start with Clean:** this option first primes the hydraulic circuit of the instrument with distilled water, and then with wash buffer. It is only recommended if the final maintenance of the previous day was omitted (i.e. the instrument was left working overnight).

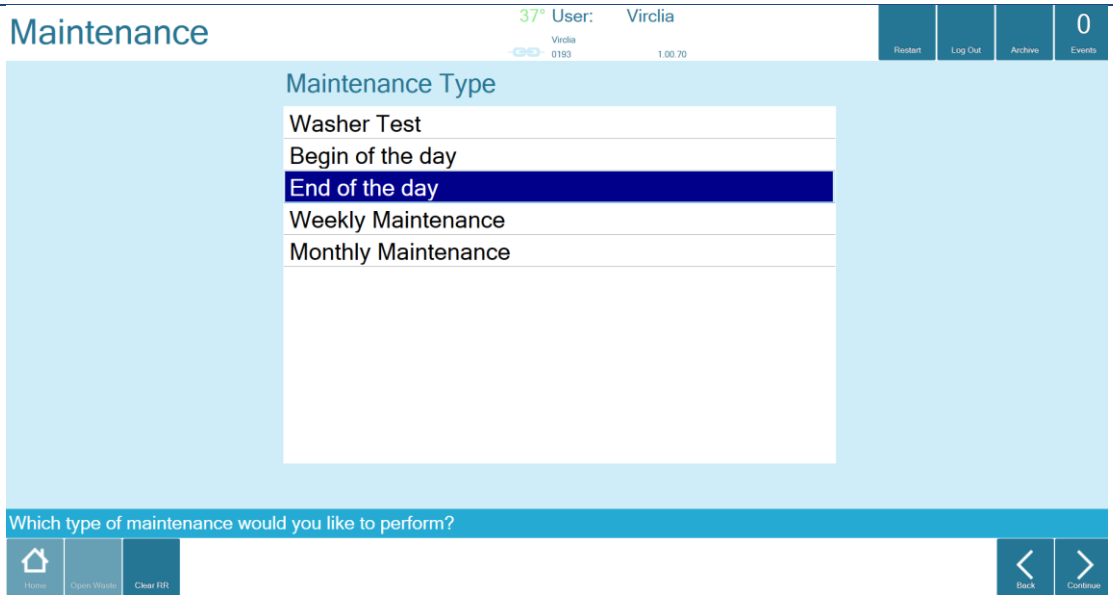
2) Through the “Maintenance” window, select the “Begin of day” option, and then click on “Continue”. The instrument performs a prime of the hydraulic circuit with distilled water, and subsequently with washing solution. This option is useful when it cannot be done through the “Start working” or “Start cleaning” options because the session is already started.



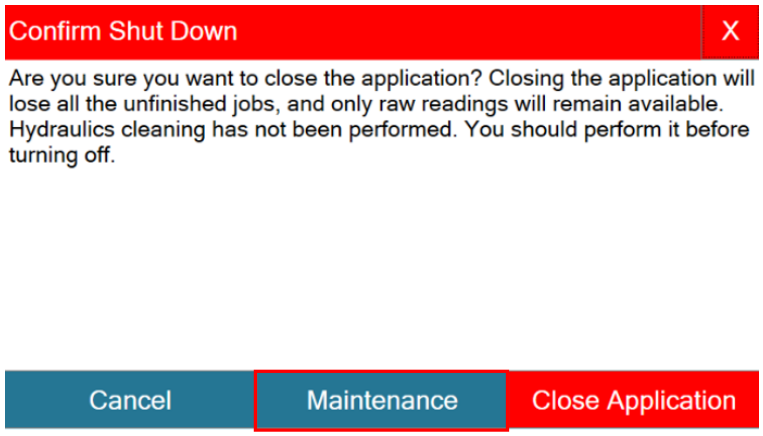
End of the day: At the end of the daily routine, before turning off the instrument, the hydraulic circuit must be primed with distilled water to avoid salt crystals growing inside the tubing and valves that could endanger the integrity of the system. If the instrument is working with continuous loading, the user has to plan this maintenance at least once per day.

To do so, fill the dedicated bottle to this purpose with distilled water and place it in its position.

Then, select the “End of the day” option and click “Continue” to prime Lotus hydraulic circuit with distilled water.



When trying to close the software, a pop-up window always appears so the user is reminded to perform the end of the day maintenance in case it was omitted. The maintenance can be performed through this window.



After priming with distilled water::

a) **Close the reagent vials.** Store according to their labels.
b) **Empty the External Waste Container.** When reconnecting the tube, make sure it is straight and free of folds, as it may hinder the emptying of the internal waste tank.
c) **Empty the Strip Waste Container.**
d) **Turn off the instrument and PC.**

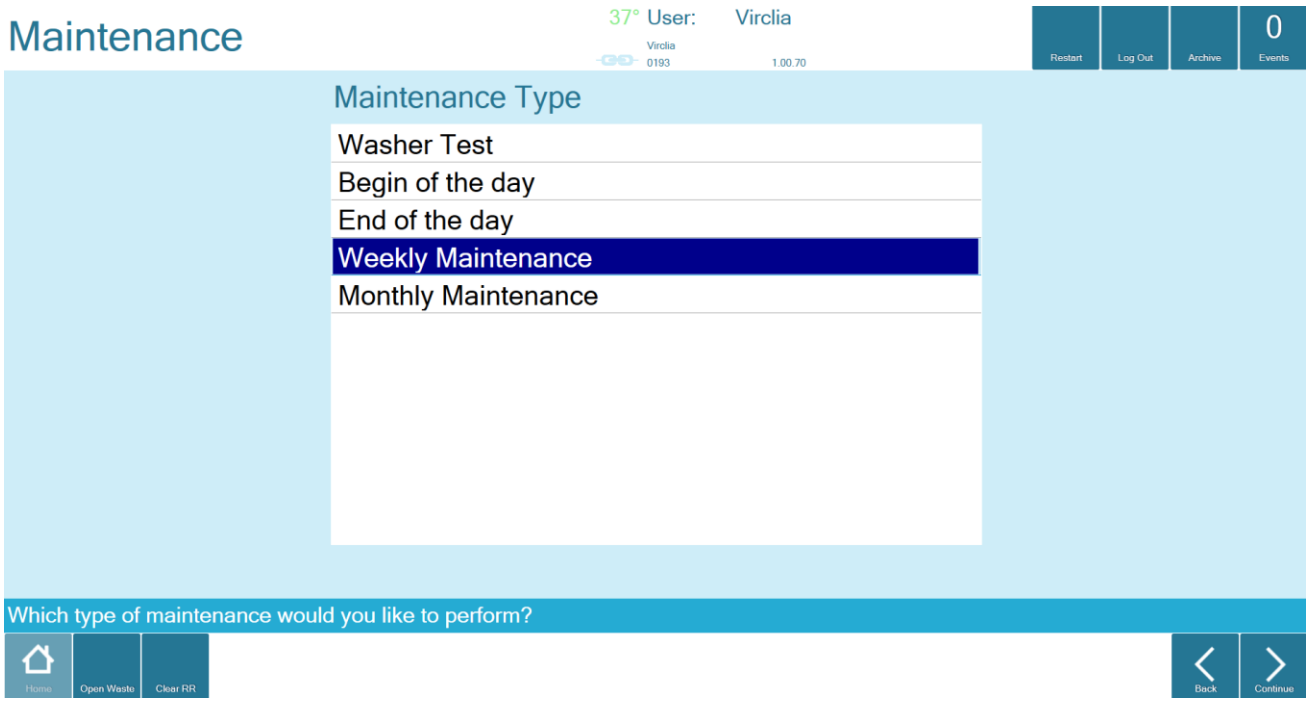
Note: any spillage should be cleaned immediately.

5.5 WEEKLY MAINTENANCE PROCEDURE

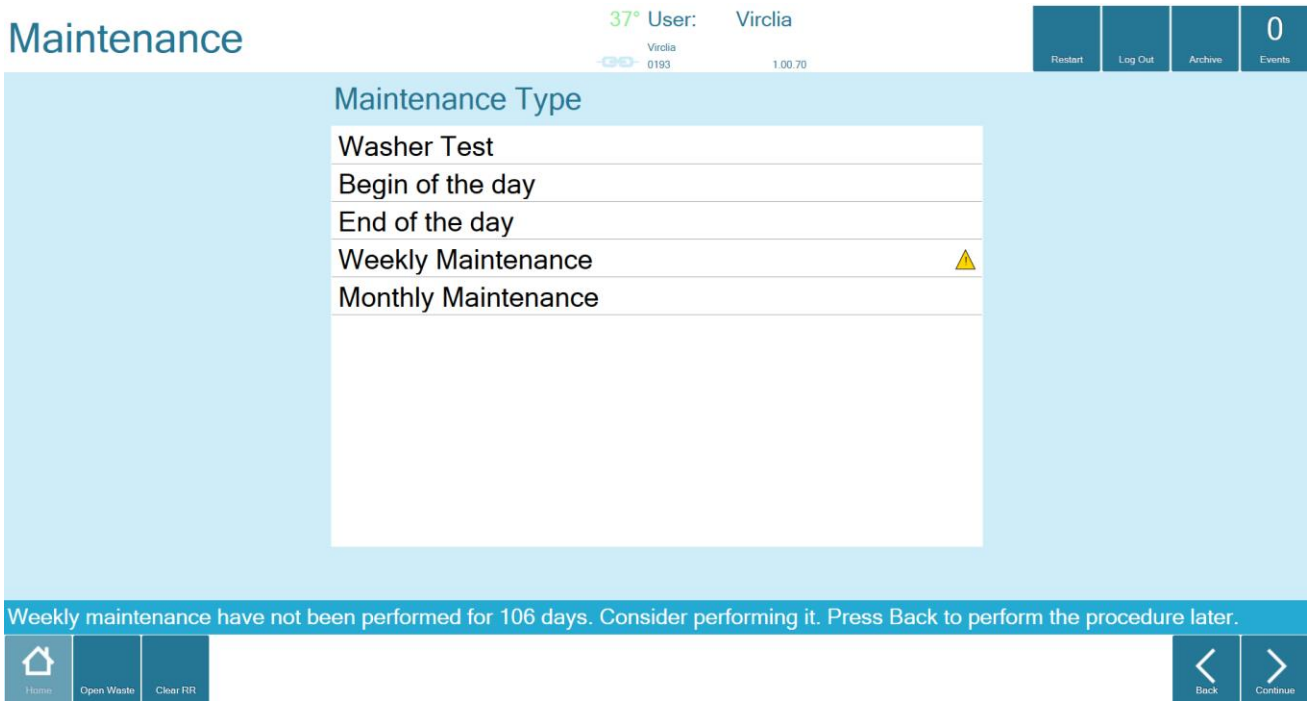
Weekly maintenance is a very straightforward and easy procedure to carry out, which includes several cleaning steps that must be performed approximately every seven days. It is recommended to perform it on the same day of the week. Precautions should be taken to ensure that no liquid comes into contact with the surfaces of the instrument during cleaning.

The aim of the weekly maintenance is to avoid adsorption of proteins inside the fluidic system, as well as the potential contamination of the instrument by environmental microorganisms.

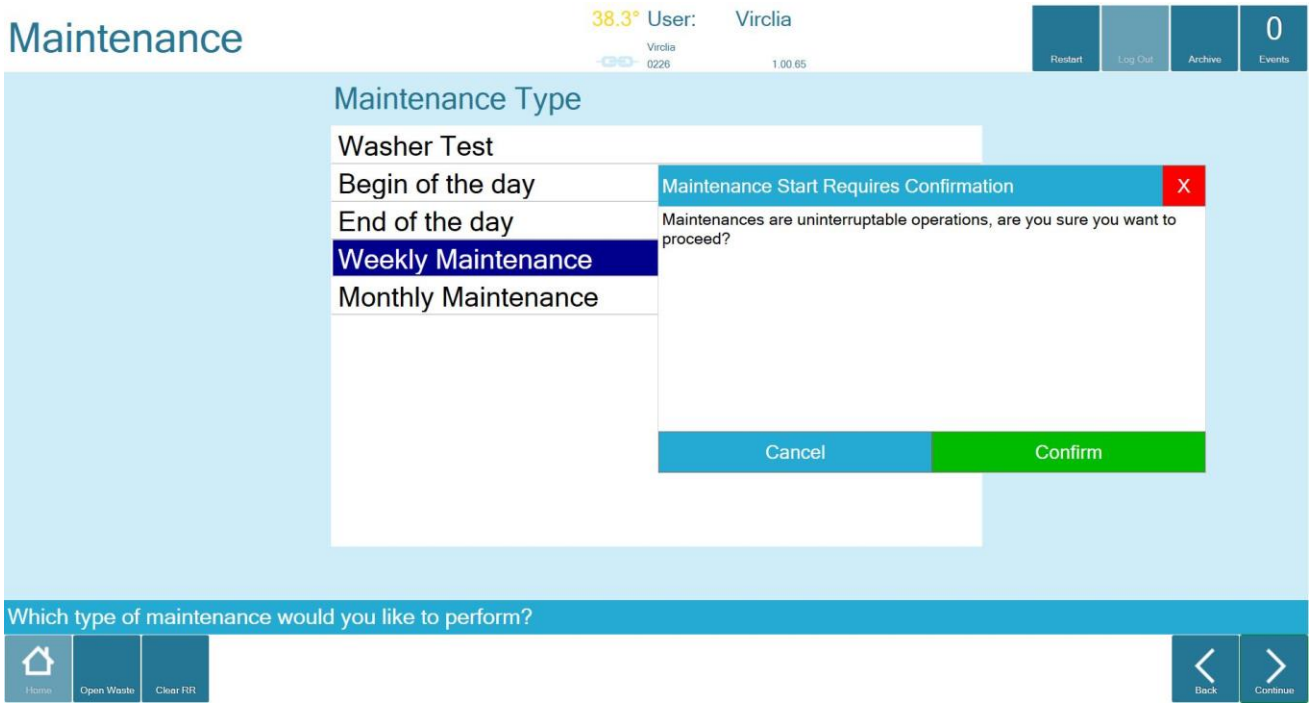
To carry it out, select the “Weekly Maintenance” option and click “Continue”



The software records when the maintenances are performed in the instrument. In case the weekly maintenance has not been performed or it is due date, an exclamation symbol will appear indicating the need for its execution.

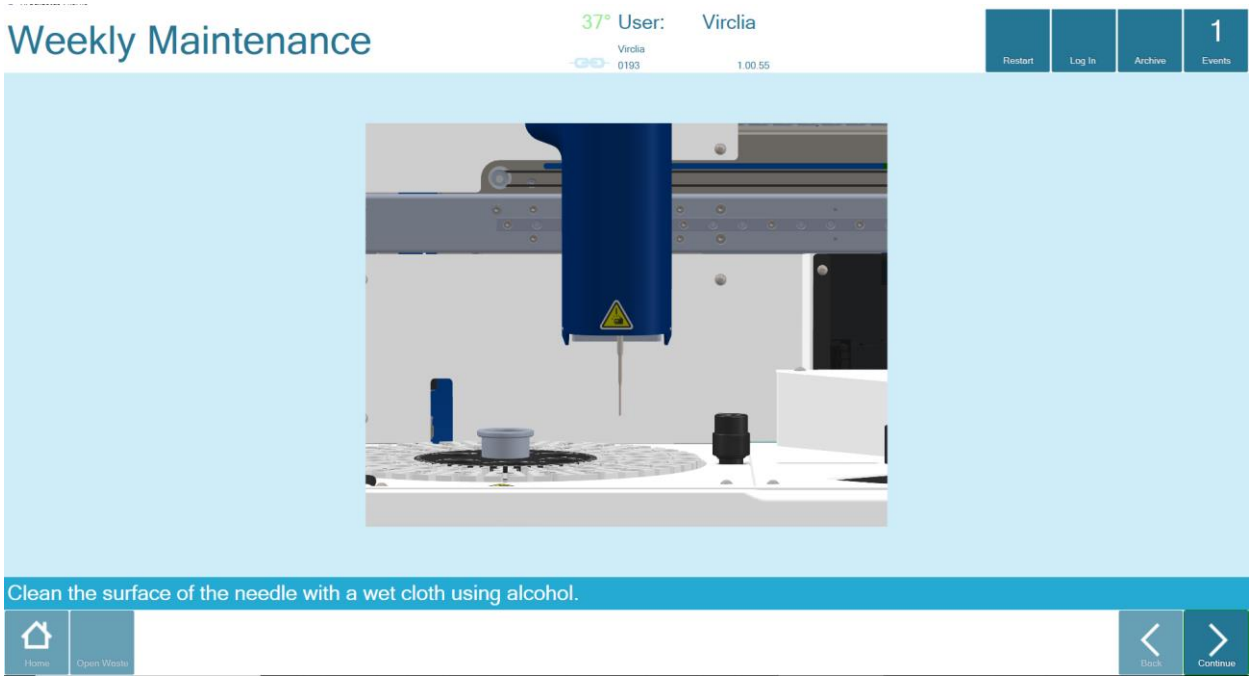


Maintenances should be carried out as one-way processes without interruption. The software shows a pop up that must be confirmed, acknowledging this matter:

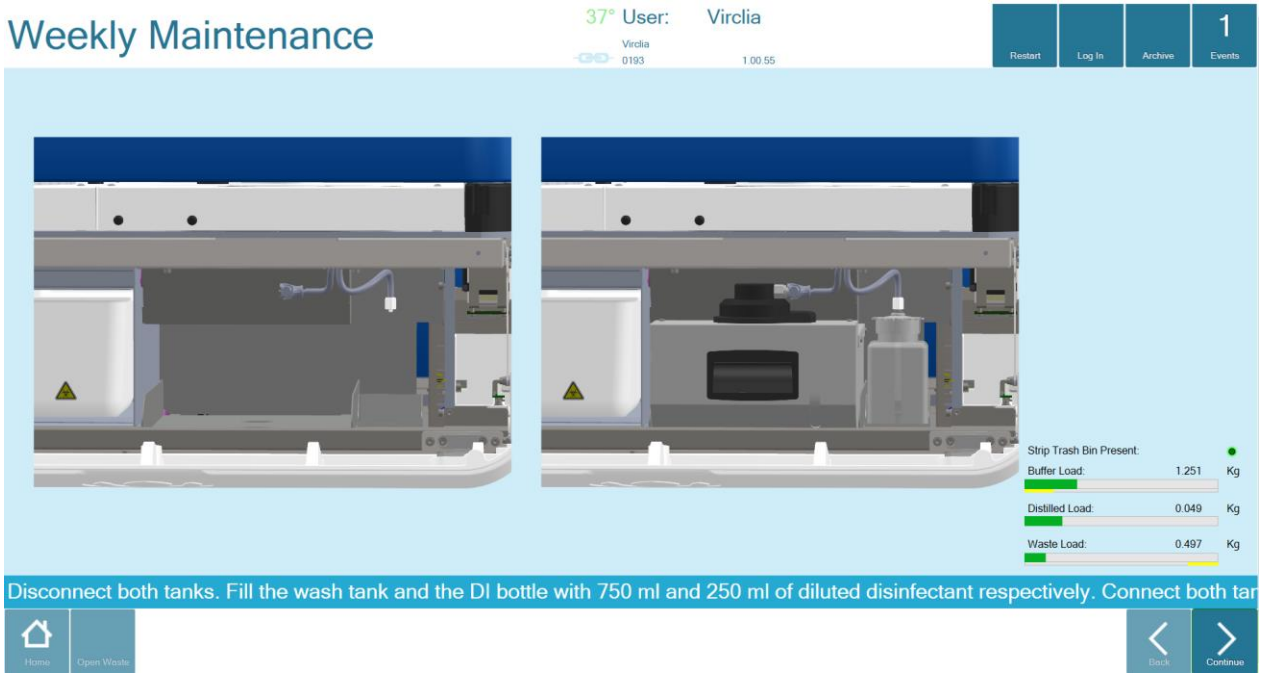


The software shows, step by step, what actions must be done to complete the weekly maintenance:

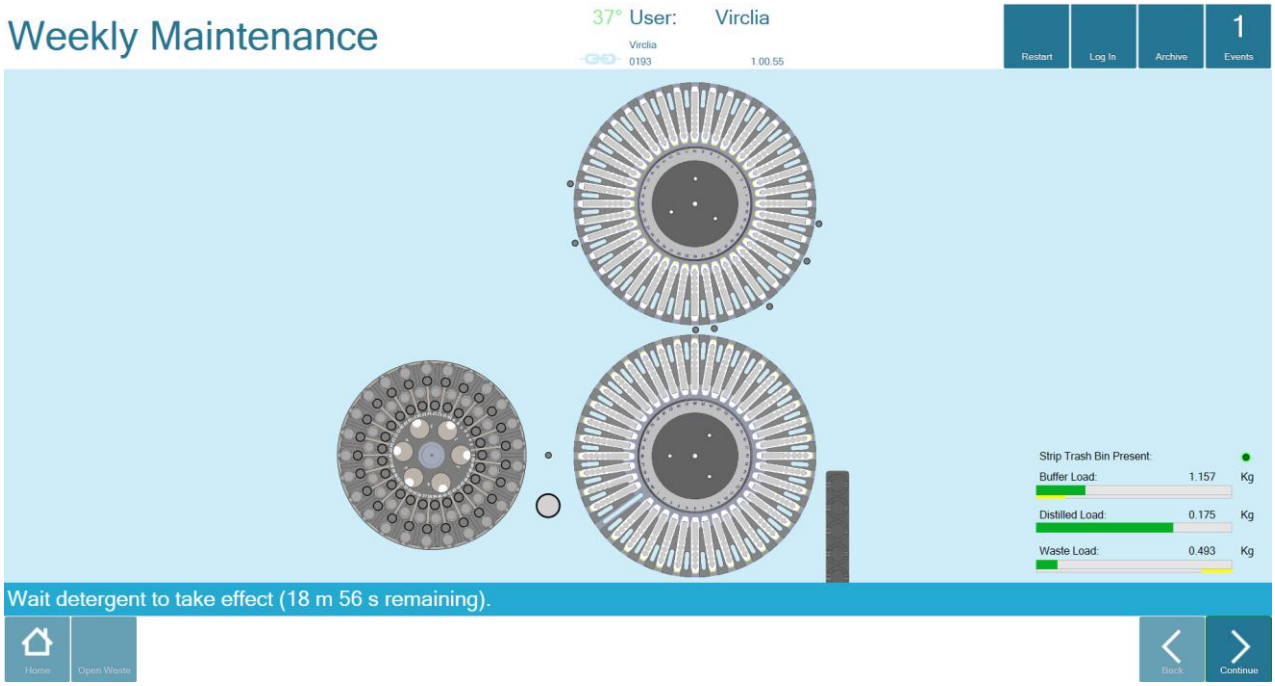
- Carefully clean the probe with a wipe moistened with alcohol.



- Vircell recommends to prepare 1 L of disinfectant detergent solution using gloves and the protective measures indicated in the safety data sheet of the product. Prepare it extemporaneously or alternatively check the expiration date assigned by the manufacturer of the disinfectant solution once diluted. Disconnect both tanks, fill them with the volume of disinfectant detergent indicated by the software, and reconnect them to the Lotus hydraulic circuit.

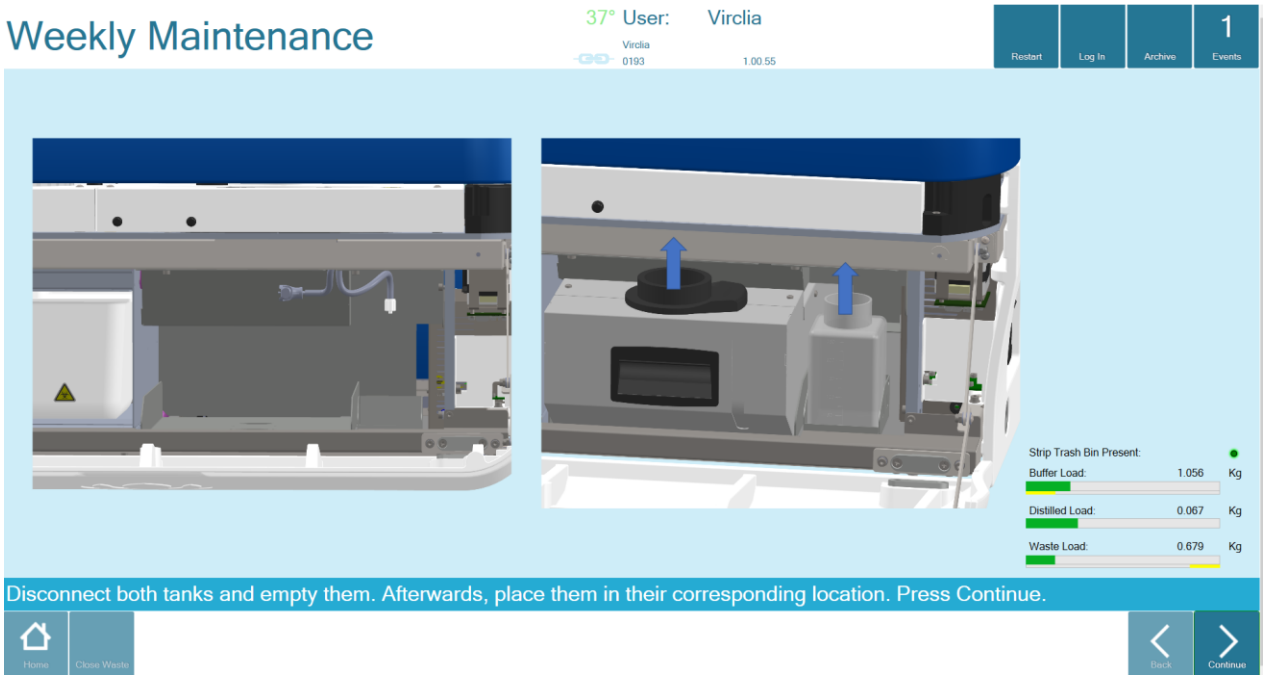


- Click on the “Continue” button to prime the hydraulic system with the disinfectant detergent solution. After finishing the priming, the solution will remain inside for 20 minutes to work. This period is monitored by the software, and once it concludes, proceed with the next maintenance step. Depending on the type of detergent used for the disinfection, follow the validation recommendations communicated by Vircell.



Finished the countdown, press “Continue” to proceed.

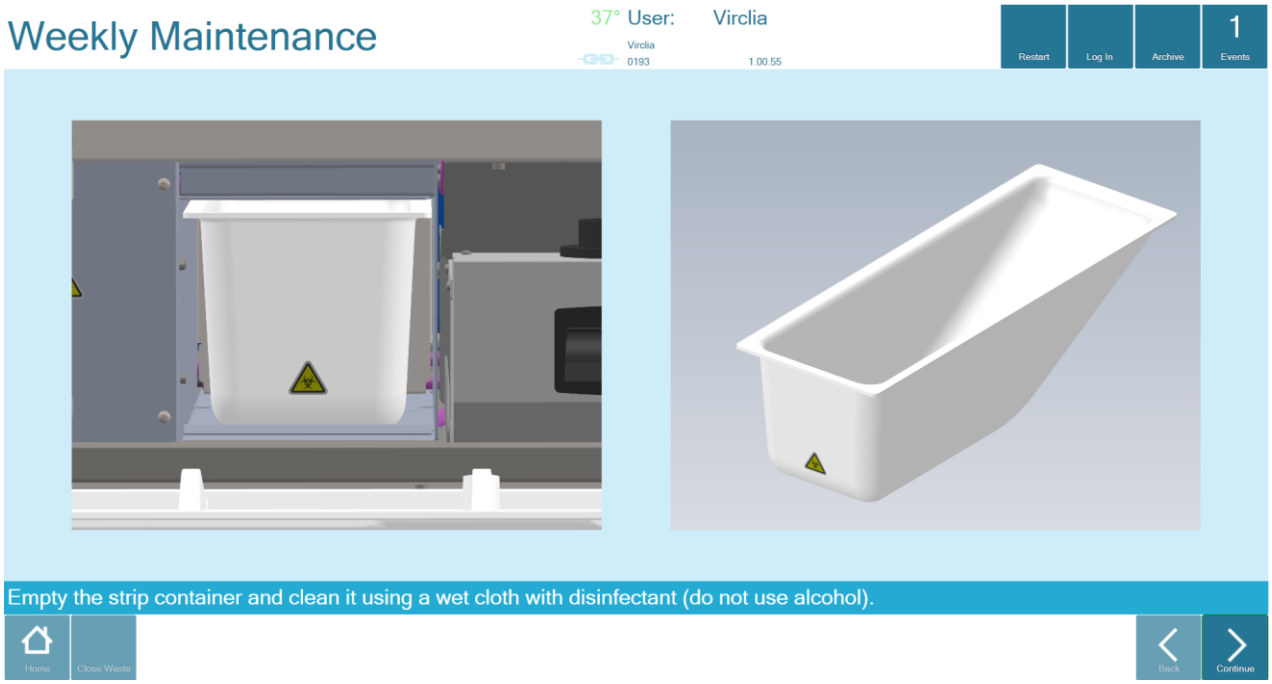
- Disconnect the bottle of distilled water, and the tank of washing solution. Discard any content that may remain inside them and rinse them thoroughly with plenty of water. Then fill them with distilled water and press “Continue”. In this way, the Lotus hydraulic circuit is primed with distilled water until clean. Once this process is complete, the software reports that both tanks can be disconnected, emptied, and then returned back to their original position.



Press “Continue” to proceed.

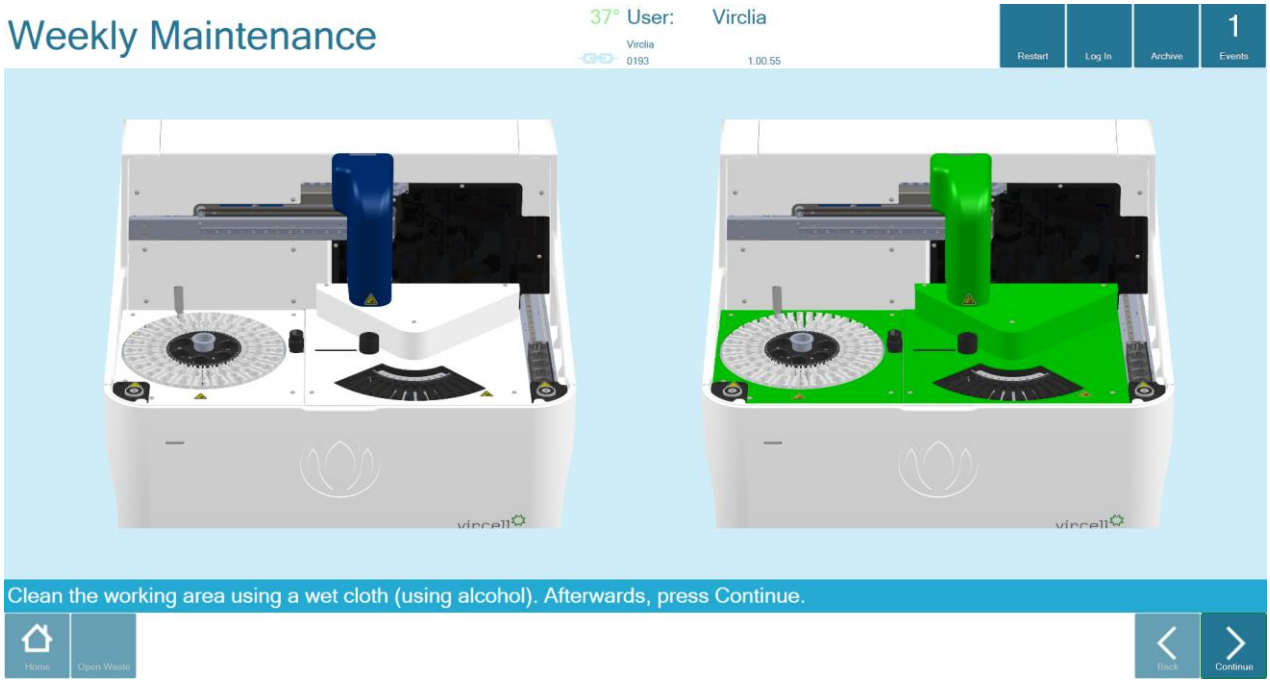
- Clean the strip waste container: inside the container there may be remains of samples and therefore there could be contact with biohazardous materials. Wearing gloves, the container should be emptied and cleaned on the inner side with a 1/10 dilution of sodium hypochlorite (bleach) in distilled water. To prepare this solution, any household bleach with a concentration ranging between 3% to 6% of sodium hypochlorite can be used. Alcohol should never be used for this purpose.

  Then rinse the container and let it dry.



Press “Continue” to proceed.

- Clean the working area with a cloth moistened with alcohol. Avoid cleaning plastic surfaces with aggressive solvents.

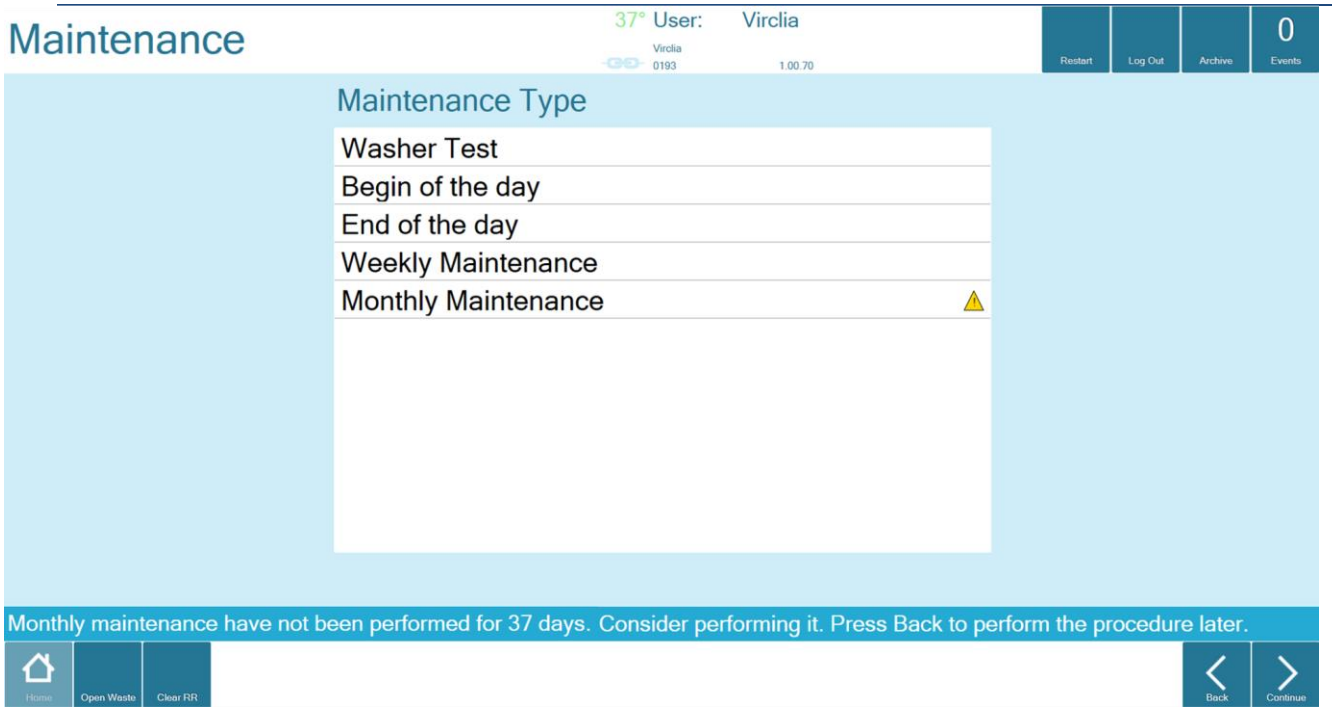


Click “Continue” to proceed.

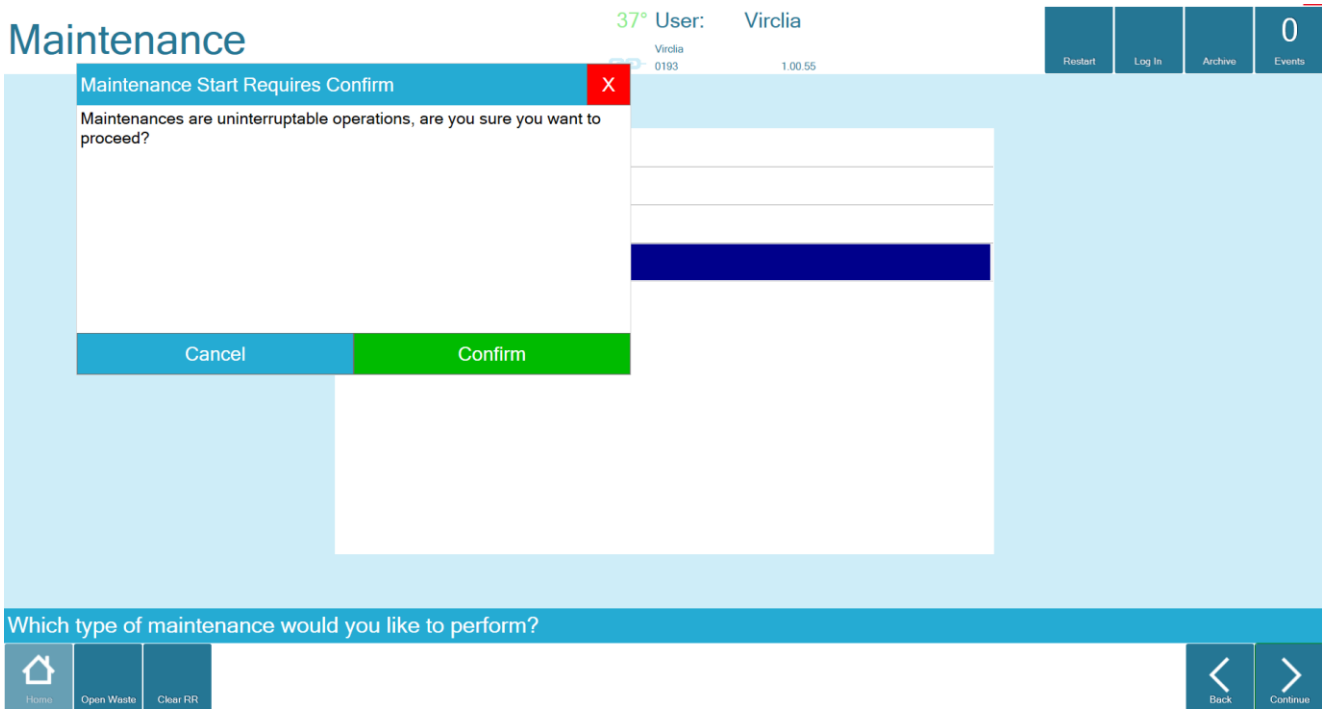
Finally, a message will appear to communicate that the weekly maintenance was completed:

5.6 MONTHLY MAINTENANCE PROCEDURE

As described in the previous sections, to perform this maintenance the option “Monthly Maintenance” has to be selected. Press “Continue” to proceed. Likewise, if the monthly maintenance has not be performed according to the schedule, an exclamation symbol will appear indicating the need for its execution.

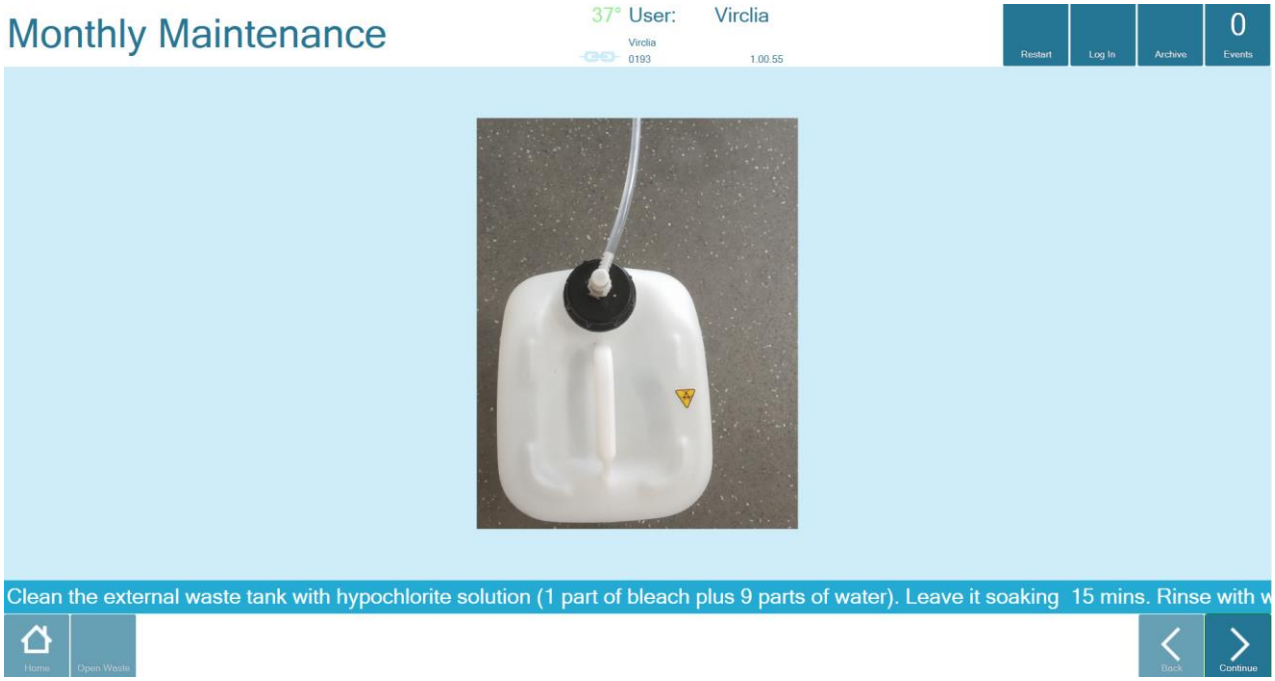


Maintenances should be carried out as one-way processes without interruption. The software shows a pop up that must be confirmed, acknowledging this matter.

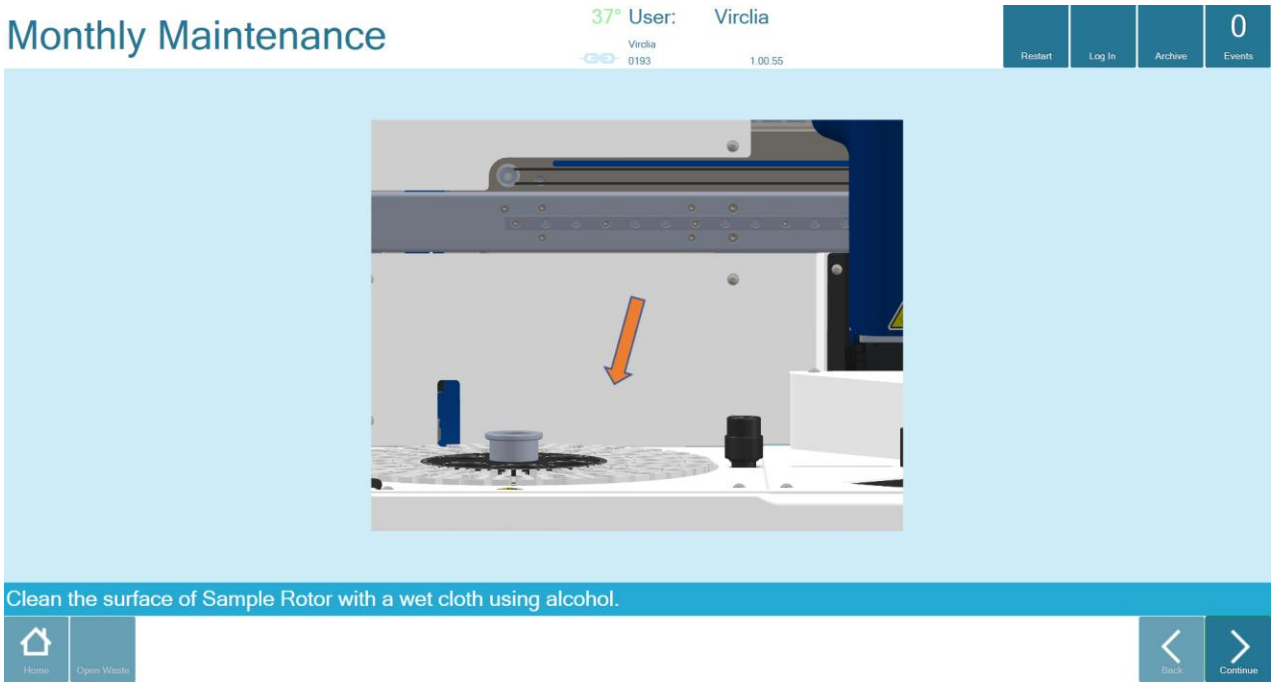


Follow the assistant´s instructions:

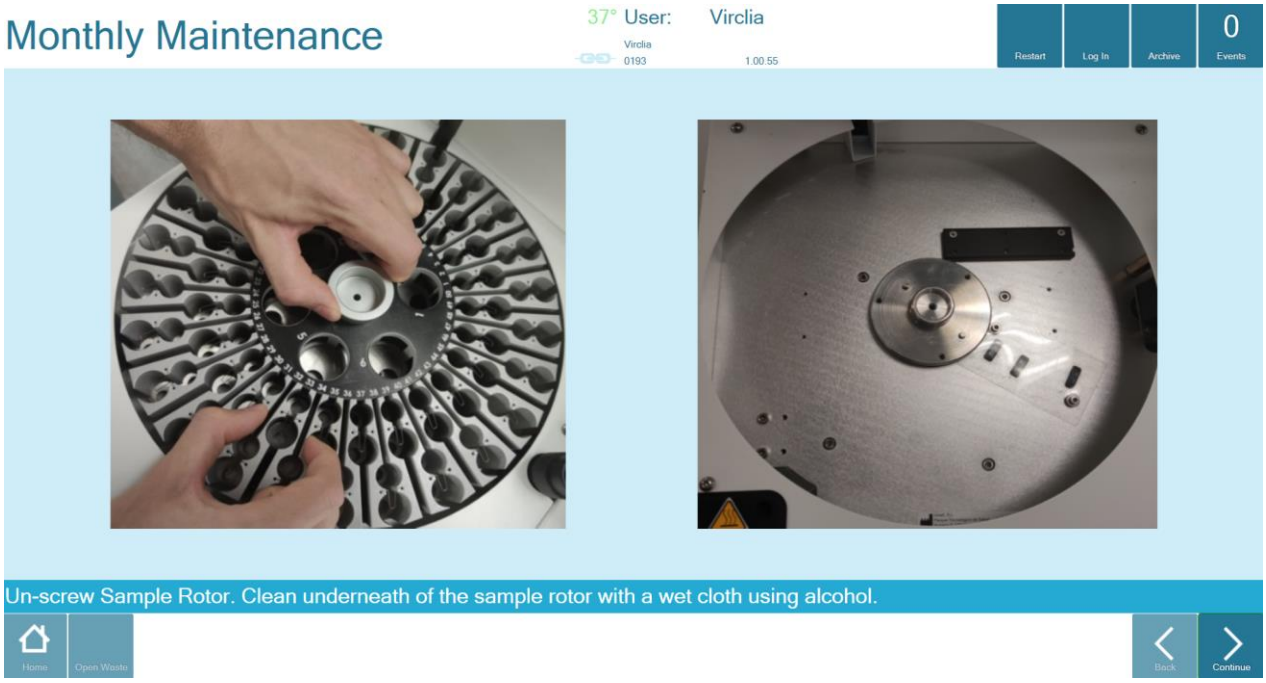
- Clean the external waste container with a sodium hypochlorite solution (1 part of bleach and 9 parts of water). To prepare this solution, any household bleach with a concentration ranging between 3% to 6% of sodium hypochlorite can be used.
- Leave the bleach solution in the container for 15 minutes. During the monthly maintenance run, the software requires confirmation that the disinfection process has been carried out.



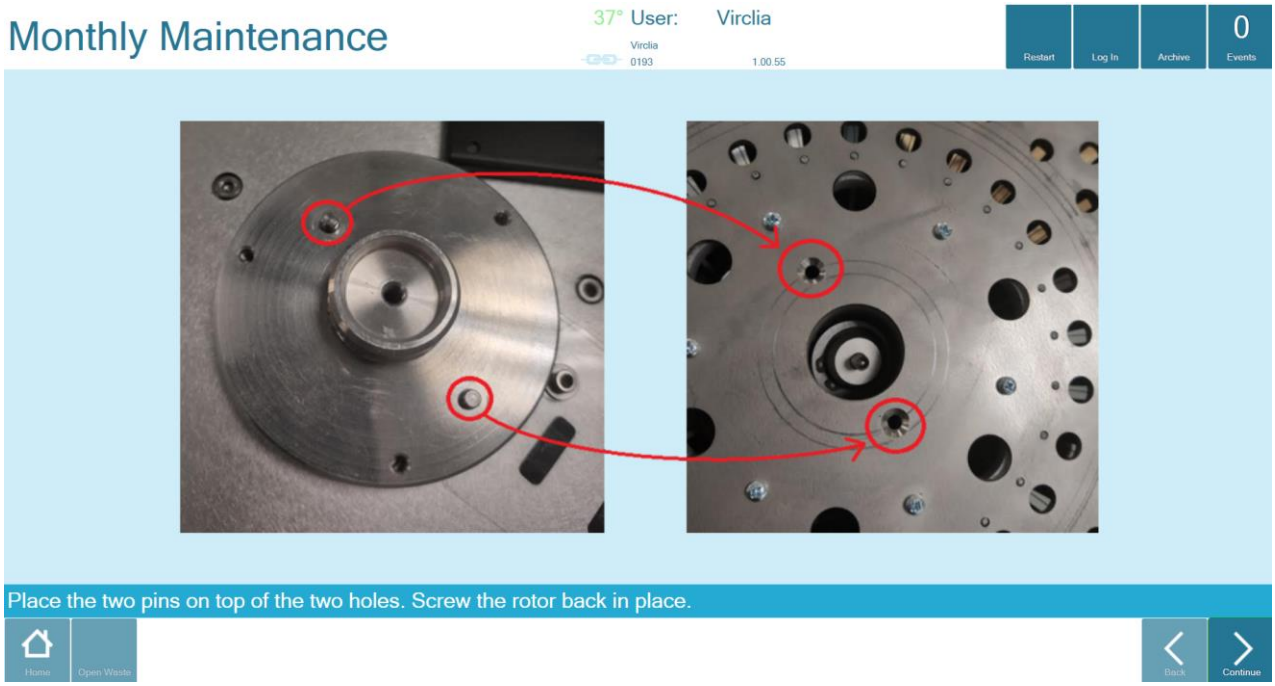
- Clean the surface of the sample rotor with a humid cloth if there is dirtiness (salts, scales, dust)



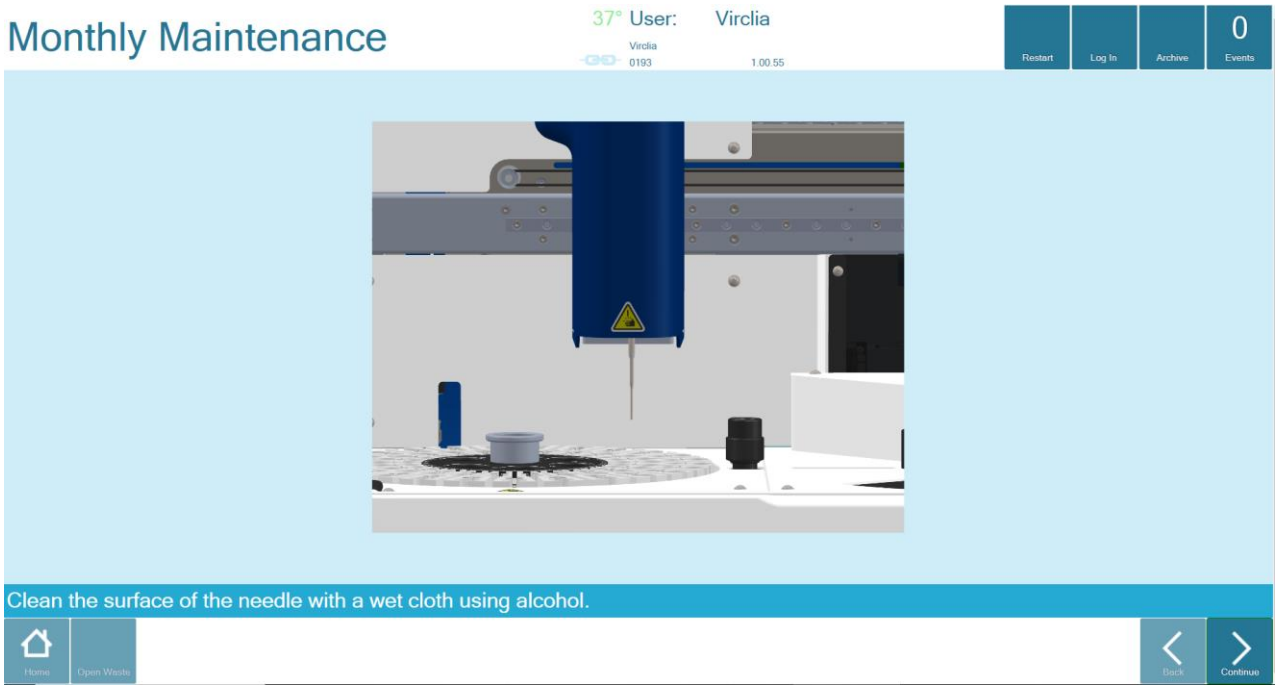
- Clean underneath the sample rotor if there are spills or dust. To do this, hold the rotor with one hand and unscrew the rotor bolt with the other hand. In this way the bottom of the rotor is accessible. Clean the surface with a cloth moistened with alcohol.



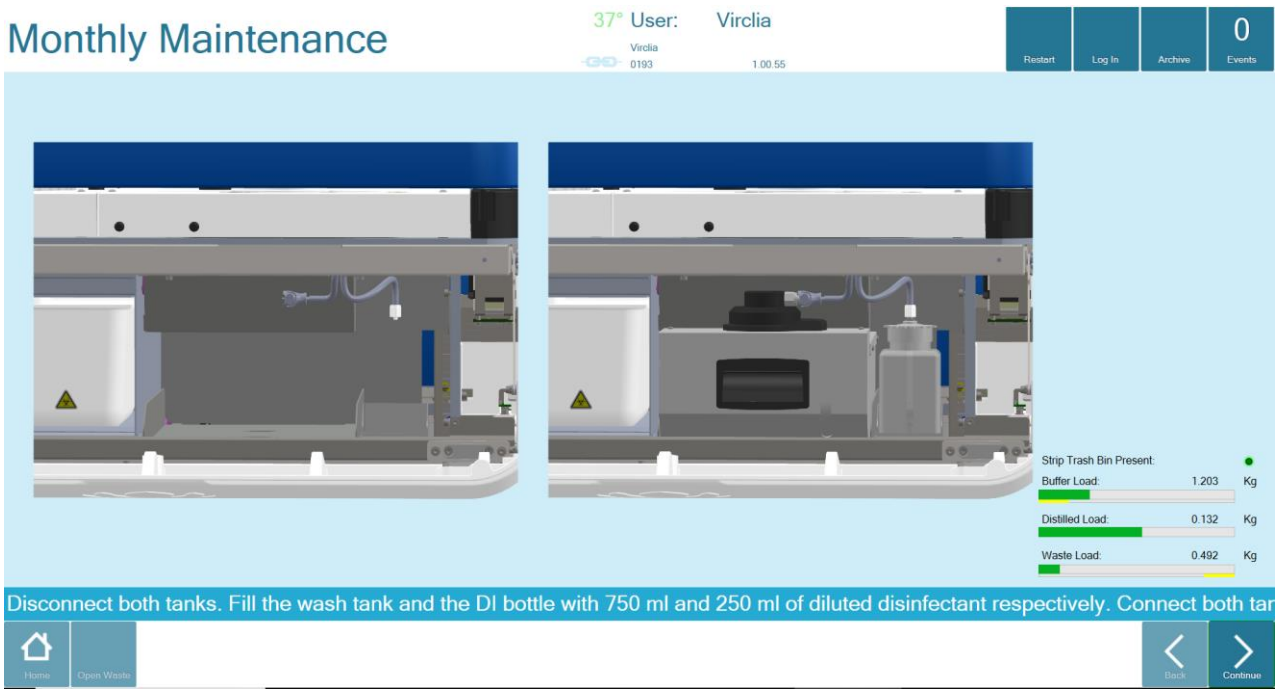
- To place the rotor back to its original position, there are two metal pins (see image below) and two holes in the bottom part of the sample rotor. To be able to correctly reattach the sample rotor, these two pins must fit in the two holes.



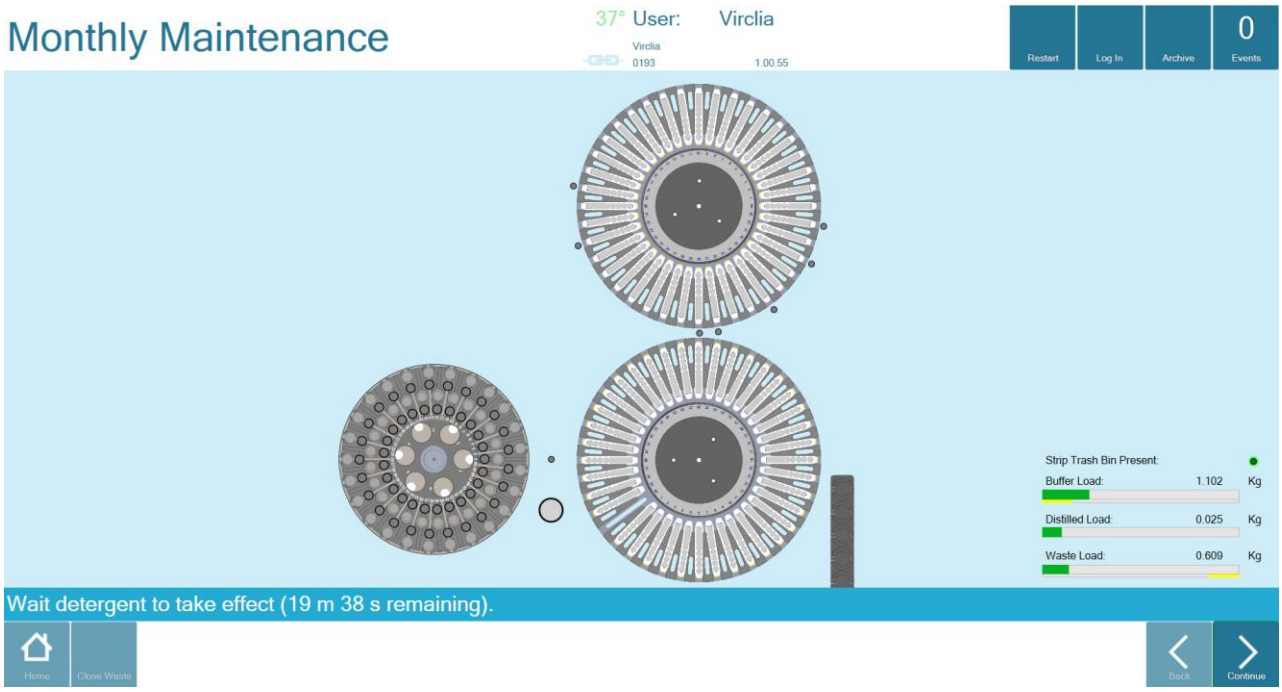
- As described in the Weekly Maintenance, clean the sample probe carefully with a cloth moistened with alcohol.



- Prepare 1 L of disinfectant detergent solution using gloves and the protective measures indicated in the safety data sheet of the product. Prepare it extemporaneously or alternatively check the expiration date assigned by the manufacturer of the disinfectant solution once diluted. Disconnect both tanks, fill them with the volume of disinfectant detergent indicated by the software, and reconnect them to the Lotus hydraulic circuit.



- Once connected, click on the “Continue” button to prime the hydraulic system with the disinfectant detergent solution. After finishing the priming, the solution must remain inside for 20 minutes to work. This time is monitored by the software, and once it concludes, you can proceed with the next maintenance step. Depending on the type of detergent used for the disinfection, follow the validation recommendations communicated by Vircell.

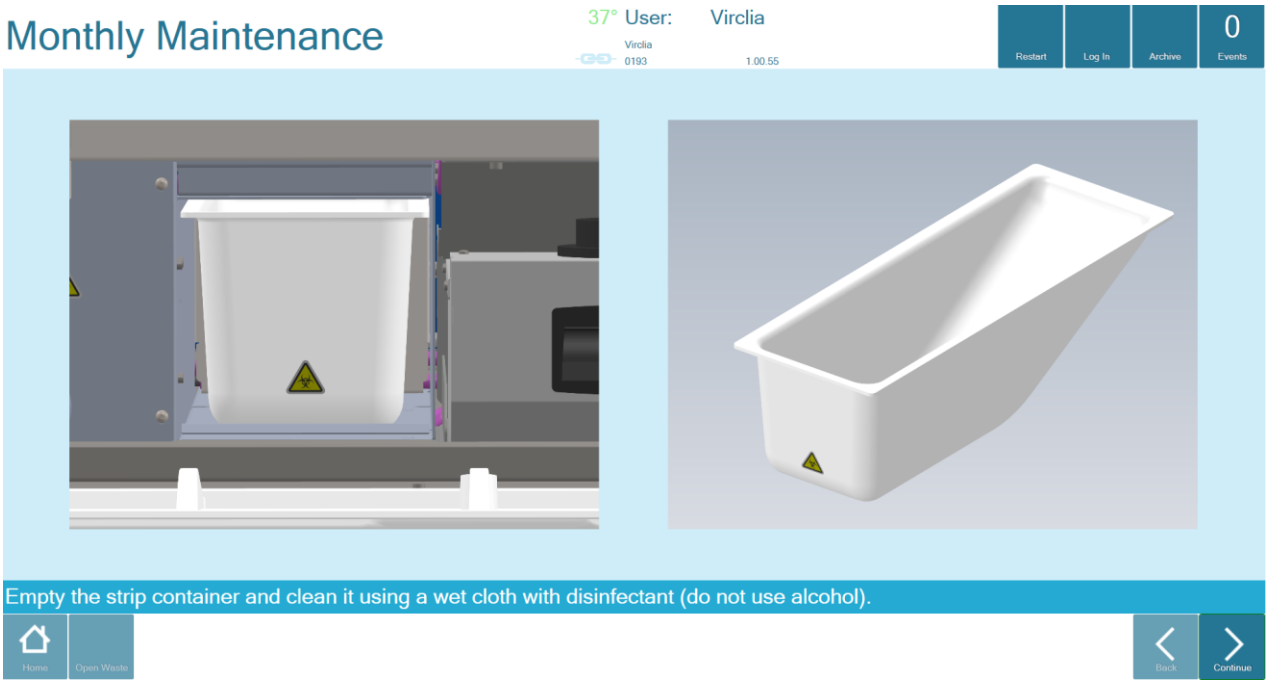


Finished the countdown, press “Continue” to proceed.

- Disconnect the bottle of distilled water, and the tank of washing solution. Discard any content that may remain inside them and rinse them thoroughly with plenty of water. Then fill them with distilled water and press “Continue”. In this way, the Lotus hydraulic circuit is primed with distilled water until clean. Once this process is complete, the software reports that both tanks can be disconnected, emptied, and then returned back to their original position.

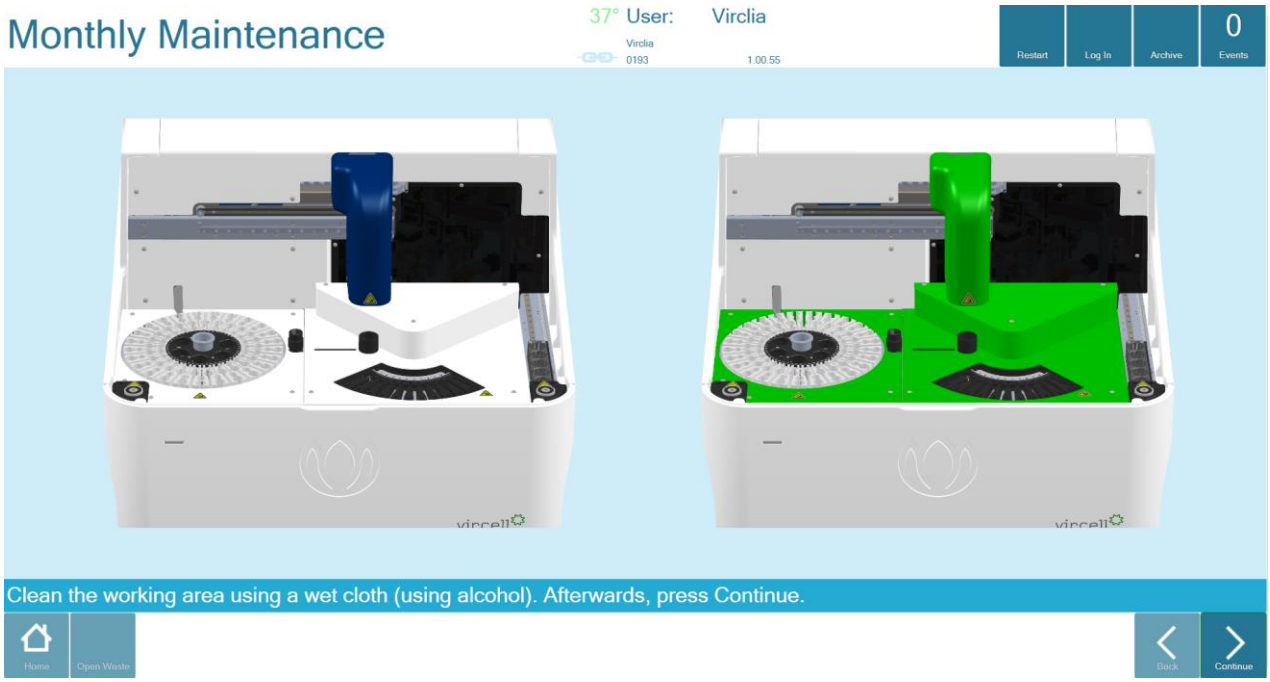
Press “Continue” to proceed.

Clean the strip waste container: inside the container there may be remains of samples and therefore there could be contact with biohazardous materials. Wearing gloves, the container should be emptied and cleaned on the inner side with a 1/10 dilution of sodium hypochlorite (bleach) in distilled water. To prepare this solution, any household bleach with a concentration ranging between 3% to 6% of sodium hypochlorite can be used. Alcohol should never be used for this purpose.



Press “Continue” to proceed.

- Clean the working area using a cloth moistened with alcohol. Avoid cleaning plastic surfaces with aggressive solvents.



Click “Continue” to finish the monthly maintenance wizard. The monthly maintenance finishes discarding the content stored inside the waste tank.

Finally, a message will appear to communicate that the monthly maintenance was completed:

5.7 BACKUP

Lotus Application has certain files that should be backed up regularly. From Vircell, we highly recommend to save a backup copy of the following folders on a regular basis (for example; once per month):

1) C:\Program Files (x86)\Lotus Application
   In this location, the containers definitions, method files and language packs (among others) can be found.
2) C:\ProgramData\Lotus
   In this location, results and log files are stored (among others).

5.8 CUSTOMIZABLE OPTIONS

The following table details the configurable messages and the default period of time.

It is recommended to contact the technical service in case any parameter needs to be modified.

Customizable options	By default
Waiting time if no volume is detected in the sample tube	15 seconds
Waiting time if no volume is detected in the predilution tube	15 seconds
Waiting time if no volume is detected in the diluent bottle	15 seconds
Expiration time for the prediluted samples	180 minutes
Periodic priming	30 minutes
Time without performing the end of the day maintenance	12 hours
Second message asking for the end of the day maintenance	1 hour
User automatic logout if no action is performed in the application	5 minutes

VIRCLIA® AUXILIARY REAGENTS-DS

REF VCMAR-DS

CE For *in vitro* diagnostic use

INTENDED PURPOSE
Auxiliary reagents for the decontamination of automated systems during the assay of VIRCLIA® kits.

MATERIALS PROVIDED
[2] VIRCLIA® DECONTAMINATION SOLUTION: 6 x 20 ml of 0.5 M sulphuric acid solution.

STORAGE AND HANDLING CONDITIONS
Store at 2-30°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-30°C.

IN-USE STABILITY
VIRCLIA® DECONTAMINATION SOLUTION: Refer to package label for expiration date (at 2-30°C).
VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS
1. For *in vitro* diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
6. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. If the kit or its components are stored in the refrigerator, please bring them at room temperature before use.
11. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
12. Keep containers for samples and reagents closed while they are not being handled.
13. Handle in aseptic conditions to avoid microbial contaminations.
14. Dispose of unused reagents and waste in accordance with all applicable regulations.
15. Use only the amount of product required for the test. Do not return the excess solution into the vial.
16. Use only with VIRCLIA® kits.
17. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Safety precautions.
Observe the following safety information. For further information a Material Safety Data Sheet is available.

Materials provided	Hazardous ingredients:	Hazard statements (CLP):
[2] VIRCLIA® DECONTAMINATION SOLUTION	Sulphuric acid CAS-No.: 7664-93-9 EC-No.: 231-639-5	H314 – Causes severe skin burns and eye damage.

Hazard statements (CLP):	H314 – Causes severe skin burns and eye damage.
Hazard pictograms (CLP):	GHS05 Corrosive

Signal word (CLP):	Danger
Precautionary statements (CLP):	P280 – Wear protective gloves/protective clothing/ eye protection/face protection. P305+P351+P338 – If in eyes: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. P303+P361+P353 – If on skin or hair: Take off immediately all contaminated clothing. Rinse skin with water or shower. P310 – Immediately call a doctor or a poison centre. P363 – Wash contaminated clothing before reuse.

PREPARATORY TREATMENT OF THE DEVICE
All reagents supplied are ready to use.

INTERNAL QUALITY CONTROL
Each batch is subjected to internal quality control (Q.C.) testing before batch release. Final Q.C. results for each particular lot are available.

SYMBOLS USED IN LABELS
IVD *In vitro* diagnostic medical device

Use-by (expiry date)

Y°C / X°C Store at x-y°C

LOT Batch code

REF Catalogue number

Consult instructions for use

Manufacturer

Current version Nr.: L-VCMAR-DS-EN-03
Date: 2022/01/17
Previous version: L-VCMAR-DS-EN-02
Updates: General Update-REACH/CLP compliance

VIRCLIA® AUXILIARY REAGENTS-EDB

REF VCMAR-EDB

CE For *in vitro* diagnostic use

INTENDED PURPOSE
Auxiliary reagents for the dilution of samples assayed with VIRCLIA® kits.

MATERIALS PROVIDED
[10] VIRCLIA® SERUM DILUENT: 2 x 10 ml of serum dilution solution: blue; phosphate buffer containing protein stabilizers and 2-Methyl-2H-isothiazol-3-one and 5-bromo-5-nitro-1,3-dioxane as preservatives. Ready to use. Contains material of animal origin.

STORAGE AND HANDLING CONDITIONS
Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY
VIRCLIA® SERUM DILUENT: Refer to package label for expiration date (at 2-8°C). VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS
1. For *in vitro* diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
6. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. If the kit or its components are stored in the refrigerator, please bring them at room temperature before use.
11. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
12. Keep containers for samples and reagents closed while they are not being handled.
13. Handle in aseptic conditions to avoid microbial contaminations.
14. Dispose of unused reagents and waste in accordance with all applicable regulations.
15. Reagents in this kit include substances of animal origin. All material should be handled and disposed as potentially infectious. Observe the local regulations for waste disposal.
16. Use only the amount of product required for the test. Do not return the excess solution into the vial.
17. Use only with VIRCLIA® kits.
18. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Safety precautions.
Observe the following safety information. For further information a Material Safety Data Sheet is available.

Materials provided	Hazardous ingredients:	Hazard statements (CLP):
[10] VIRCLIA® SERUM DILUENT	2-Methyl-2H-isothiazol-3-one CAS-No.: 2682-20-4 EC-No.: 220-239-6	H317 – May cause an allergic skin reaction.

Hazard statements (CLP):	H317 – May cause an allergic skin reaction.
Hazard pictograms (CLP):	 GHS07 Health hazard/ Hazardous to the ozone layer
Signal word (CLP):	Warning
Precautionary statements (CLP):	P261 – Avoid breathing dust/fume/ gas/mist/vapours/spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P302+P352 – If on skin: Wash with plenty of water. P321 – Specific treatment (see supplemental first aid instruction on this label). P333+P313 – If skin irritation or rash occurs: Get medical advice/ attention.

PREPARATORY TREATMENT OF THE DEVICE
All reagents supplied are ready to use.

INTERNAL QUALITY CONTROL
Each batch is subjected to internal quality control (Q.C.) testing before batch release. Final Q.C. results for each particular lot are available.

SYMBOLS USED IN LABELS

IVD	*In vitro* diagnostic medical device
	Use-by (expiry date)
Y°C / X°C	Store at x-y°C
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
	Manufacturer

Current version Nr.: L-VCMAR-EDB-EN-02
Date: 2022/01/17
Previous version: L-VCMAR-EDB-EN-01
Updates: General Update-REACH/CLP compliance

VIRCLIA® AUXILIARY REAGENTS-W1

REF VCMAR-W1

CE For *in vitro* diagnostic use

INTENDED PURPOSE
Auxiliary reagents for the washing and reagent dilution steps of VIRCLIA® kits.

MATERIALS PROVIDED
[1] VIRCLIA® WASHING SOLUTION (20x): 1 L of 20x washing solution: a phosphate buffer containing Tween®-20 and Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1).

STORAGE AND HANDLING CONDITIONS
Store at 2-8°C. Do not use the kit reagents beyond the expiration date. This will be valid only if reagents are stored closed and at 2-8°C.

IN-USE STABILITY
VIRCLIA® WASHING SOLUTION diluted (1x): 4 months at 2-8°C.
VIRCELL, S.L. does not accept responsibility for the mishandling of the reagents included in the kit.

WARNINGS AND PRECAUTIONS
1. For *in vitro* diagnostic use only. For professional use only.
2. The product should be limited to personnel who have been trained in the technique.
3. The user of this kit is advised to carefully read and understand the package insert. Strict adherence to the protocol is necessary to obtain reliable test results.
4. Use only protocols described in this insert. Conditions other than specified may give erroneous results.
5. Wear personal protective equipment when handling samples and reagents. Wash hands properly after handling the samples and reagents. All procedures must be carried out in accordance with the approved safety standards.
6. Use only clean, preferably disposable material.
7. Never pipette by mouth.
8. Do not use in the event of damage to the package.
9. Do not use the kit after expiration date.
10. If the kit or its components are stored in the refrigerator, please bring them at room temperature before use.
11. Do not leave the reagents at temperature different to the recommended longer than absolutely necessary.
12. Keep containers for samples and reagents closed while they are not being handled.
13. Handle in aseptic conditions to avoid microbial contaminations.
14. Dispose of unused reagents and waste in accordance with all applicable regulations.
15. Use only the amount of product required for the test. Do not return the excess solution into the vial.
16. Use only with VIRCLIA® kits.
17. Any serious incident that occurs in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

Safety precautions.
Observe the following safety information. For further information a Material Safety Data Sheet is available.

Materials provided	Hazardous ingredients:	Hazard statements (CLP):
[1] VIRCLIA® WASHING SOLUTION (20x)	Reaction mass of 5-chloro-2-methyl-2H-isothiazol-3-one and 2-methyl-2H-isothiazol-3-one (3:1) CAS-No.: 55965-84-9	H317 – May cause an allergic skin reaction.

Hazard statements (CLP):	H317 – May cause an allergic skin reaction.
Hazard pictograms (CLP):	 GHS07 Health hazard/ Hazardous to the ozone layer
Signal word (CLP):	Warning
Precautionary statements (CLP):	P261 – Avoid breathing dust/fume/ gas/mist/vapours/spray. P272 – Contaminated work clothing should not be allowed out of the workplace. P280 – Wear protective gloves/protective clothing/eye protection/face protection. P302+P352 – If on skin: Wash with plenty of water. P321 – Specific treatment (see supplemental first aid instruction on this label). P333+P313 – If skin irritation or rash occurs: Get medical advice/ attention.

PREPARATORY TREATMENT OF THE DEVICE
VIRCLIA® WASHING SOLUTION must be prepared in advance. Fill 1000 ml of VIRCLIA® WASHING SOLUTION (20x) up to 20 litres with distilled water. Should salt crystals form in the washing concentrate during storage, warm the solution to 37°C before diluting.

INTERNAL QUALITY CONTROL
Each batch is subjected to internal quality control (Q.C.) testing before batch release. Final Q.C. results for each particular lot are available.

SYMBOLS USED IN LABELS

IVD	*In vitro* diagnostic medical device
	Use-by (expiry date)
Y°C X°C	Store at x-y°C
LOT	Batch code
REF	Catalogue number
	Consult instructions for use
	Manufacturer

Current version Nr.: L-VCMAR-W1-EN-02
Date: 2021/12/27
Previous version: L-VCMAR-W1-EN-01
Updates: General Update-REACH/CLP compliance