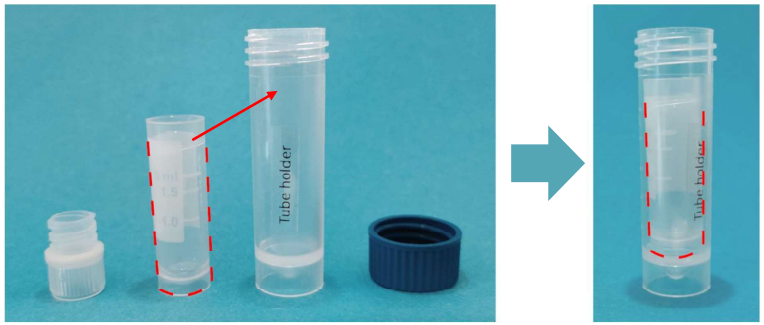
How to: Sterile solution

This last solution contains cells-free water, complemented with preservatives. Measurements of this sample should lead to counts/ ml as low as possible, giving a cleanliness assessment of the device.

Warning: *Single use only. Discard the aliquots after having done 1x Prime et 2x Analysis water. Attempting to run validation procedures with incorrect volumes risks introducing air in the system which may affect the results.*

Sterile validation procedure

1. Install the manual sampling device.
2. Tear open an alcohol-based pad and clean the sampling needle (wipe from the top down).
3. Wait for the needle to dry.
4. Mount the waste solution tubes **or** the Washstation
5. Launch 2x "Clean sampling device" procedure **or** 1x "Clean Sampling Device with Washstation"
6. Remove the tube **or** Washstation once done.
7. Use the default gate template, already recorded in the device, according to the cartridge type (TCC, ICC, LDC). (Gate Settings / Check Box)
8. Take one of the "Sterile solution" aliquots , remove its cap and put the aliquot's tube inside one of the tube holders. **Do not transfer the liquid itself but place the whole aliquot's tube inside the tube holder.**



9. Load the tube holder, containing the aliquot's tube, on the manual sampling device.
10. Launch 1x "Prime" procedure from the BactoSense's manual mode.
11. Launch 2x "Water analysis" procedures from the BactoSense's manual mode and remove the tube holder once done.
12. Discard the "S terile solution" aliquot and store the tube holder.
13. Disable the Validation Mode. (Maintenance / Instrument Validation)
14. To pass the sterile validation the analysis results must be as follows:

	TCC	ICC	LDC
Sterile Water	TCC <100 / mL	ICC <100 / mL	ICC <100 / mL TCC < 3000 / mL

Additional information

If the results look contaminated during one or several validation steps, it is recommended to use the BactoSense's Cleaning kit before attempting a new validation procedure. Using the cleaning kit will put back the device to a perfectly clean state, which is ideal to perform and succeed any validation step.

bNovate TECHNOLOGIES

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User Manual Validation Kit BactoSense

General information

This kit is suitable to validate the BactoSense. The chemistry contained in the kit is designed to be used after having measured drinking water samples only. When measured, samples other than water, such as wastewater, beverage, culture medium, etc., are the exclusive responsibility of the user. The validation kit efficiency might be reduced and cannot be guaranteed in such cases. One kit is designed to perform approximately 10 validation operations. The kit is stable one year if stored under correct conditions (2-8°C away from light).

Safety information

Read the user manual
Read this manual before use

Wear protective gloves
Wear protective gloves during handling. In case of skin contact, rinse immediately and abundantly with fresh water. In case of pain or irritation, consult a doctor.

Wear protective glasses
Wear safety glasses

Warning

Validation kit content

Item	Quantity	Validation function	Notes
Beads solution	2	Check the alignment of optical components	Before starting to use beads solution, ensure that the device has been previously cleaned correctly. Considered empty once the level goes below 1.5 mL.
Reference solution	2	Check the fluorescence signals of the dye(s) and the overall sample preparation	Before starting to use the reference solution, ensure that the device has been previously cleaned correctly. Considered empty once the level goes below 1.5 mL.
Sterile solution aliquot	10	Check the cleanliness of the fluidic	Before starting to use the sterile solution, ensure that the device has been previously cleaned correctly. Single use only: discard each aliquot after having done 1x Prime and 2x Water analysis.
Tube holder	2	Hold the sterile solution aliquots	Re-usable.
Waste solution	2	Collect back-wash	Considered full once the level reaches 5 mL .
Alcohol-based pads	10	Clean the sampling needle's outer surface	Always start cleaning from the top of the sampling needle to avoid introducing any lint on the needle tip.

When to use the validation kit

Any time a validation of the BactoSense is needed:

- After a major transportation (plane, train, etc.)
- After a long period out of use (>2 months)
- After nonsense results

Please refer to this document to properly validate the instrument. This kit contains different compounds, each of which have a specific function during the validation.
It is recommended to perform the **validation** in the following **sequence: Beads / Reference / Sterile solution**

How to: Beads solution

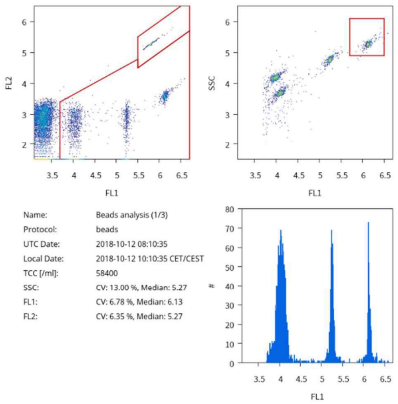
This solution contains different types of micro and nano beads (containing specific fluorophores) with various sizes and fluorescent intensities. These beads check the quality of the optic alignment for the 3 channels (FL1, FL2 and SSC).

Warning: *Discard the 5 mL tubes once they have less than 1.5 mL of liquid in them. Attempting to run validation procedures with incorrect volumes risks introducing air in the system which may affect the results.*

Beads validation procedure

1. Login as Admin
2. Install the manual sampling device.
3. Tear open an alcohol-based pad and clean the sampling needle (wipe from the top down).
4. Wait for the needle to dry.
5. Mount the "Waste solution" tubes **or** the Washstation
6. Launch 2x "Clean sampling device" procedure **or** 1x "Clean Sampling Device with Washstation"
7. Remove the tube **or** Washstation once done.
8. Enable the "Validation mode". (Maintenance / Instrument Validation)
9. The default gate "Fit template" must be used. (Gate Settings / Check Box)
10. Take one of the "Beads solution" tubes and shake it vigorously for ten seconds **or** use a vortex mixer, if available.
11. Once homogenized, mount the tube into the sampling device.
12. Launch 1x "Prime" procedure from the BactoSense's manual mode.
13. Launch 2x "Beads analysis" procedures from the BactoSense's manual mode and remove the tube once done.
14. To pass the optical validation the analysis results must be as follows:

TCC [/mL]:	**[54'000 ; 72'000]**
SSC:	**CV < 25%, Median: [4.7 ; 6.3]**
FL1:	**CV < 15%, Median: [5.7 ; 6.5]**
FL2:	**CV < 20%, Median: [5.1 ; 5.7]**



How to: Reference solution

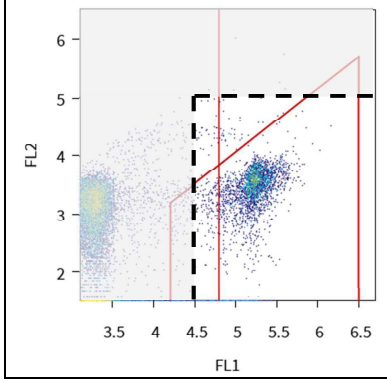
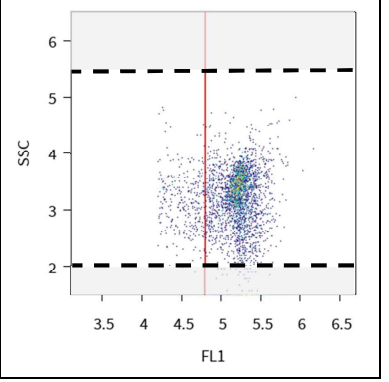
In order to test the dye(s) ability to fluoresce, this reference solution contains the cell's population(s) which appear within specific coordinates into the dot plots (FL1/FL2 & FL1/SSC).

Warning: *Discard the 5 mL tubes once they have less than 1.5 mL of liquid in them. Attempting to run validation procedures with incorrect volumes risks introducing air in the system which may affect the results.*

Reference validation procedure

1. Install the manual sampling device.
2. Tear open an alcohol-based pad and clean the sampling needle (wipe from the top down).
3. Wait for the needle to dry.
4. Mount the waste solution tubes **or** the Washstation
5. Launch 2x "Clean sampling device" procedure **or** 1x "Clean Sampling Device with Washstation"
6. Remove the tube **or** Washstation once done.
7. Use the default gate template, already recorded in the device, according to the cartridge type (TCC, ICC, LDC). (Gate Settings / Check Box)
8. Put on one of the "Reference solution" tubes.
9. Launch 1x "Prime" procedure from the BactoSense's manual mode.
10. Launch 2x "Water analysis" procedures from the BactoSense's manual mode
11. Remove the tube once done.
12. To pass the reference validation, the main part of cluster in both TCC (for TCC cartridge only) and ICC (for ICC and LDC cartridges) should appear as follows:

Dotplot A:	**> 4.5 in the FL1 axis**	**and**	**< 5 in the FL2 axis**
Dotplot B:	**> 2 in the SSC axis**	**and**	**< 5.5 in the SSC axis**

Dotplot A (FL1/FL2)	Dotplot B (FL1/SSC)
	

Note: This reference solution relies on bacteria which are, by essence, subject to evolution even if properly stored. Bacterial populations (TCC, ICC, DCC) can be sensitive to environmental variations and may therefore appear a bit differently from one kit to another or over time. But, as long as the population of interest stays in the mentioned coordinates, this validation procedure remains reliable. The above example shows the main population of a reference solution measured with an ICC cartridge. TCC and LDC cartridges will produce different results and use different gating but that is not relevant for this test. Accurate counts/ml are not relevant in this test neither.