

Checking and calibration of serological pipettes for precise volume setting

The permissible volume tolerance for 9 ml diluent for performing serial dilutions for plate counts is $\pm 200 \mu\text{l}$, or $\pm 2.2 \%$, according to ISO 6887-1:2017. The Inlabtec Serial Diluter uses standard 10 ml serological pipettes as the volume determining element. The dispensing accuracy of the device and thus compliance with the normative specifications is therefore determined by the accuracy of the volume indications (scale) on the pipettes used.

The specified accuracy of the scale of plastic serological pipettes is usually $\pm 2 \%$. Depending on the manufacturer's production process, the specified tolerance of $\pm 2 \%$ is more or less utilized or even exceeded if quality assurance is inadequate. However, the tolerance between pipettes of the same batch/lot is usually low, as the same production conditions are applied.

If a pipette is installed in the Serial Diluter, a constant additional volume of approx. 50 - 100 μl is added to the set pipette volume due to its design. This additional volume depends on the shape fit of the serological pipette with connection piece of the tubing set. Depending on the accuracy of the pipette, the additional volume may cause the tolerated maximum volume of 9.2 ml for the diluent to be exceeded.

By checking and, if necessary, calibrating the scaling of the pipettes used of a batch/lot, the required accuracy of the serial diluter can be achieved easily. If the deviation of the pipette scale from the setpoint is known, the sensor can be correctly positioned on the scale to maintain accuracy with a deviation of $\pm 2.2 \%$.

Testing and calibration of the pipettes in use

First, check the accuracy of the scale of the pipettes used to determine the correct value on the pipette scale for 9 ml. Procedure:

Step	Carry out	Comments
1	Install 10 ml serological pipette of a specific batch/lot in the Serial Diluter.	
2	Adjust the sensor of the Serial Diluter so that exactly 9 ml pipette volume is displayed on the scale.	
3	Fill three Serial Dilution Bags. Weigh the filled bags individually and protocol the measured values.	First fill a bag to wet the tubes of the Serial Diluter. Do not use this bag any further.
4	Repeat steps 1 – 3 for pipette 2 and 3 of the same batch/delivery	Use pipettes from the same delivery/batch/lot.
5	Enter the measured values in the Excel template "Pipette_Calibration" to determine the calibration value for the tested pipettes for 9 ml.	

Verification of the pipette testing and calibration

The determined pipette scale calibration value is checked with at least one other pipette of the same batch. Procedure:

Step	Carry out	Comments
1	Install a 10 ml pipette from the checked batch/ lot in the Serial Diluter.	
2	Set the pipette volume to the determined calibration value for 9 ml, e.g. 8.7 ml.	
3	Fill six Serial Dilution Bags and weigh the filled bags individually. Protocol the measured values.	First fill a bag to wet the tubes of the Serial Diluter. Do not use this bag any further.
4	Enter measured values per pipette in the Excel template "Verification Pipettes" and determine maximum deviation.	
5	Repeat steps 1 – 4 for further pipettes of the same batch/ lot, e.g. for in total three pipettes, or according to internal requirements.	

Comments

- The volume dispensed by the Serial Diluter is defined by the volume adjusted using the pipette scale.
- Calibration of the scale of the pipettes used ensures the required accuracy for serial dilutions and reduces the testing and correction effort in routine operation to a minimum.
- Normally, one calibration and verification per batch/lot of the 10 ml pipettes used is sufficient.
- A calibration must be carried out if pipettes from another manufacturer or another batch/ lot from the same manufacturer is used.
- To keep the calibration effort low, deliveries of pipettes should consist of one batch/lot (note accordingly when ordering).
- It is recommended to attach the calibration value for the pipettes tested and used to the Serial Diluter (Fig. 1).



Fig. 1: Serial Diluter with indication of the pipettes in use and their calibration value for 9 ml. So, for these pipettes N242.1, the sensor must be set to 8.7 ml to obtain 9 ml volume per dilution.