

# pH Measurements of Agar Plates

## With InLab<sup>®</sup> Surface Pro-ISM Sensor

This application note explains why the InLab Surface Pro-ISM sensor from METTLER TOLEDO is the ideal solution for measuring the pH of agar plates. USP 41 <1117> recommends pH as a test parameter in the quality control of growth media, such as agar plates. Their solid gelatinous nature and protein-containing composition, however, makes it challenging to measure pH accurately and reliably using a conventional sensor. Here you will also find practical tips on achieving accurate results and prolonging sensor life.

Using the right pH sensor along with good measuring practices are key factors in obtaining consistent media quality and complying with regulations. For pH testing, USP 41 <1117> recommends the usage of a flat probe. The pH of every batch of medium produced is confirmed after it has cooled down to room temperature (20-25°C). Refrigerated purchased media need to be brought up to room temperature for measurement. The pH of media should be in a range of  $\pm 0.2$  of the value indicated by the manufacturer, unless a wider range is admissible by a validated method.



**METTLER TOLEDO**

## The Right Sensor Choice: InLab Surface Pro-ISM

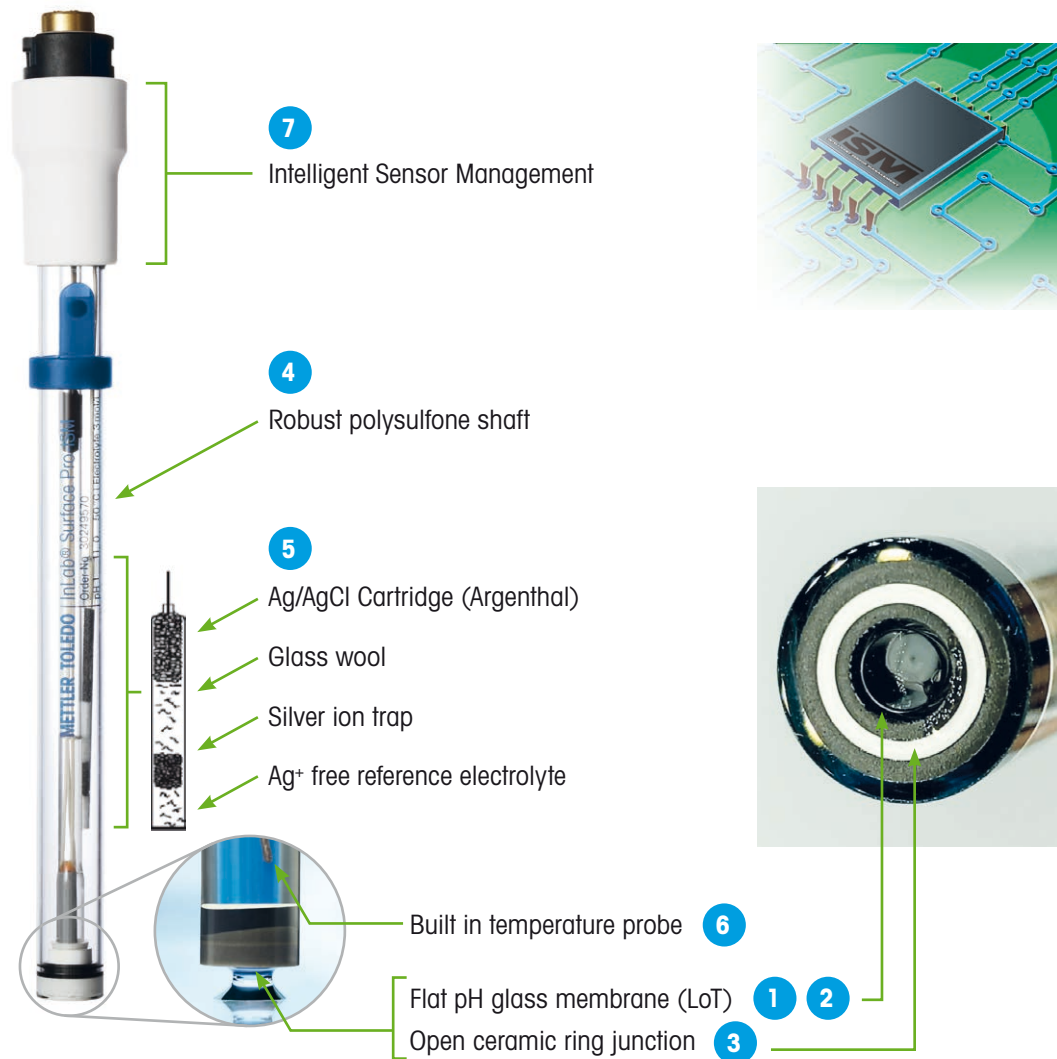


Figure 1: InLab Surface Pro-ISM, features infographic.

No.	Challenge	Solution	Description
1	Measuring pH of small, solid surfaces	Flat pH membrane	Ensures that the junction is in contact with the sample which is necessary to get a measurement.
2	Unstable liquid junction potential	LoT glass membrane	Has a low resistance glass membrane, making the sensor highly pH-sensitive.
3	Longer response time	Open ceramic ring junction	Ensures relatively fast outflow of electrolyte reducing the measurement delay.
4	Sensor shaft prone to damage	Robust polysulfone shaft	Ensures better chemical and mechanical resistance. Makes the sensor easy to handle, improving its durability.
5	Leaching of silver ions from the reference element (Ag wire) causes the following: 1. Unstable readings 2. Precipitate between Ag and proteins and sulfides (from nutrient media) block and contaminate the junction. This results in reduced outflow of reference electrolyte, leading to fluctuations in readings.	ARGENTHAL™ reference system	AgCl granulate-filled cartridge, supplies the silver ions for the chemical reaction on the conducting wire. The silver ion trap stops the Ag <sup>+</sup> ions from discharging into the electrolyte solution, thus preventing precipitation and contamination of the junction. Therefore, stable readings are obtained as compared to the conventional sensor with Ag/AgCl reference system.
6	Inconsistencies in results caused by varying temperatures	Integrated temperature probe (and ATC functionality of METTER TOLEDO pH meters)	pH is temperature dependent. With the integrated temperature probe, the sample temperature is noted. During calibration, the pH value is compensated for temperature effects to give more accurate slope.
7	Secure and easy data handling	ISM® (Intelligent Sensor Management)	Sensor keeps the track of important information such as latest calibration data, maximum temperature exposure of the sensor along with sensor information, providing maximum traceability and data security. It also helps in autorecognition of sensor upon connecting to the meter.

Table 1: Features and advantages of InLab Surface Pro-ISM.

## Good Measuring Practices for Consistent and High-Quality Results

### A. Calibration

- Calibrate the system regularly using MT buffers, at least once a day.
- Verify the calibration by measuring a pH buffer of known pH value between the calibrations.
- Use at least two pH buffers for calibration bracketing the expected sample pH e.g. use pH buffers 4.01 and 7.00 for calibration if the sample pH is expected to be at 5.5.
- A slope of 95-105% and an offset of  $0 \text{ mV} \pm 30 \text{ mV}$  ensures good state of electrode and hence reliable measurements.
- Always use fresh pH buffers of high quality and never re-use them.
- To obtain accurate results, pH buffers and sample solutions must be at same temperature.

### B. Measurement

- Prepare the agar plates as per the respective protocol and verify the pH after sterilization as changes in pH may occur. In case of commercially purchased media, it is not mandatory.
- Gently place the tip of InLab Surface Pro-ISM on the surface of the agar medium. Record the pH using automatic end-point.
- Perform measurements of the samples at the required temperature in triplicates.
- After every measurement, thoroughly clean the pH sensor using deionized water. Use mild soapy water or ethanol if required. In that case, rinse off again using deionized water to remove traces of the solvent.
- Do not rub the sensor surface; always blot-dry with paper tissue to remove any excess water.
- Do not expose the sensor to harsh acidic or alkaline chemicals. The pH range defined for InLab Surface Pro-ISM is 1-11 units and thus covers the pH requirements for agar media.

### C. Maintenance

- Periodically recondition the sensor in 0.01M HCl based on sensor performance. The frequency of reconditioning depends on the number of samples analyzed per day and the age of the sensor. Old sensors require more frequent conditioning compared to new sensors.
- Re-calibrate the sensor after reconditioning.

### D. Storage

- Between measurements or overnight, keep the sensor in the wetting cap filled with InLab Storage solution.
- Never store the sensor dry or in distilled water, as this affects the pH-sensitive glass membrane, leading to a shortened lifetime.

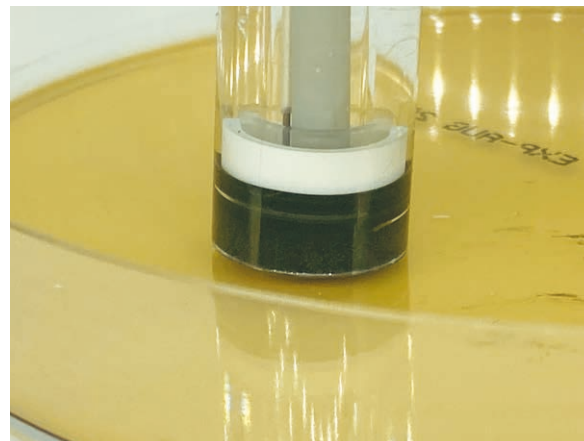


Figure 3: Measurement using InLab Surface Pro-ISM on a potato dextrose agar plate.

## Results and Discussion

Three different types of agar plates were measured using InLab Surface Pro-ISM pH sensor, applying good practices as outlined in the previous chapter.

Sample	pH value		Standard deviation	Average time (s)
	Expected	Obtained		
Solid sample nature	7.3	7.22	0.03	22
Potato dextrose agar plate	5.6	5.55	0.03	45
R2A agar plate	7.20	7.20	0.04	33

Table 2: pH values of agar plates measured with InLab Surface Pro-ISM sensor.

The measurement system used yielded repeatable results in agreement with the expected values and with acceptable response time. A standard deviation of less than 0.05 pH and an average response time of less than 60 seconds was observed for the agar plate samples measured.

## Conclusion

The specialist sensor, InLab Surface Pro-ISM proves to be the right choice for measuring the pH of agar plates in compliance with the guidelines laid down in standards and regulations. Implementing best practices for calibration and measurement along with proper sensor maintenance ensures better results throughout the lifetime of the sensor.

## References

- United States Pharmacopeia, 41<sup>st</sup> Edition. Physical Tests and Determinations <1117> "Microbiological Best Laboratory Practices".
- Reasoner, D.J., and Geldreich, E.E. 1979. Paper No. N7, Abstracts of the Annual Meeting of The American Society for Microbiology, Washington, D. C.
- Isenberg, H.D. Clinical Microbiology Procedures Handbook. 2<sup>nd</sup> Edition, 2016.
- The Official Compendia of Standards. USP-NF. United States Pharmacopeial Convention, Rockville, MD.

## Further information from METTLER TOLEDO

- Electrode handling videos on YouTube:  
▶ [www.mt.com/pHLab\\_ElectrodeHandlingVideos](http://www.mt.com/pHLab_ElectrodeHandlingVideos)
- Comprehensive range of pH meters, electrodes, solutions, and accessories:  
▶ [www.mt.com/pH](http://www.mt.com/pH)
- White paper about USP <791>:  
▶ [www.mt.com/pHLab\\_USP791](http://www.mt.com/pHLab_USP791)
- pH in small sample application note:  
▶ [www.mt.com/pHLab\\_SmallSamples](http://www.mt.com/pHLab_SmallSamples)

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