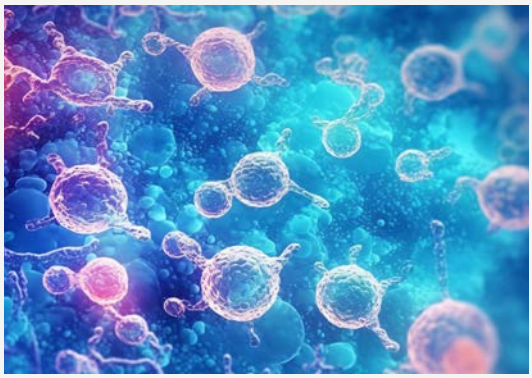
VISERION
RAMAN SPECTROSCOPY

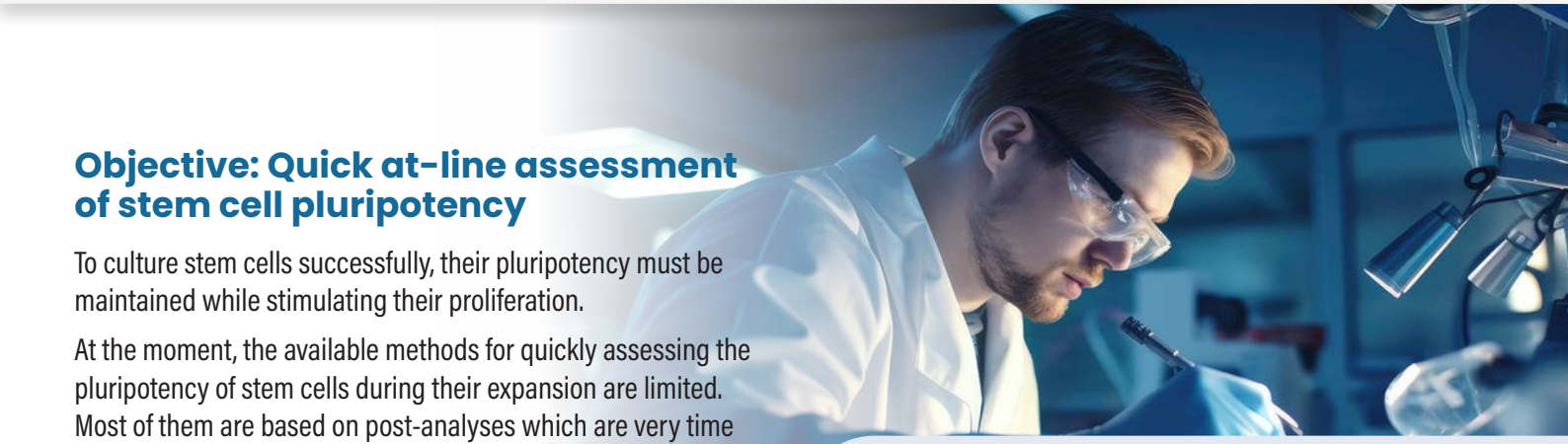
CHAUVIN ARNOUX
INDATECH

Biotech



Determination of stem cell pluripotency using Raman spectroscopy

In the last few decades, pluripotent stem cells (PSCs) have attracted massive interest from the pharmaceutical sector as a future therapeutic solution for treating chronic diseases. Research in this field has focused mainly on increasing PSCs production. It is therefore necessary to ensure robust monitoring and control of the processes when transitioning from the laboratory to production scale.



Objective: Quick at-line assessment of stem cell pluripotency

To culture stem cells successfully, their pluripotency must be maintained while stimulating their proliferation.

At the moment, the available methods for quickly assessing the pluripotency of stem cells during their expansion are limited. Most of them are based on post-analyses which are very time consuming and are subject to significant variations due to the operator.

In order to assess this critical attribute, we propose using Raman spectroscopy as an analytical tool for classifying PSCs pluripotency.

Viserion: the solution for in/at-line analysis of critical parameters

Indatech offers a unique inspection solution capable of checking both the physical and chemical characteristics of liquids. Viserion can be used for quick, non-destructive at-line analyses.

It can:

- Perform simultaneous measurements using up to four channels
- Provide information on the chemical composition of complex liquids
- Perform very quick acquisitions (< 0.5 s in isopropanol)
- Analyze finished products through plastic packaging or glass container

At-line measurement of stem cell pluripotency: how?

The culture medium samples measured come from six different experiments.

Stem cell expansion is performed with daily renewal of the culture media. A sample of this culture medium (without cells) is then taken for analysis by means of Raman spectroscopy (see Figure 01). In total, 158 samples were measured, including 51 with bad pluripotency.

The Raman spectra were collected using a Viserion® 785 nm multichannel analyzer covering a spectral range from 250 to 3,200 cm-1. The samples were analyzed with the «Vial Analyzer» using 10 mm quartz cuvettes.

The design of the Vial Analyzer allows robust, reproducible analysis of the sample because the probe head is fixed to the support containing the sample. The spectra were recording using a 100 s integration time, with three accumulations. The use of ≥ 3 accumulations makes it possible to automatically filter the peaks in the spectra resulting from cosmic rays.

This function is implemented in the Viserion Ready® software.

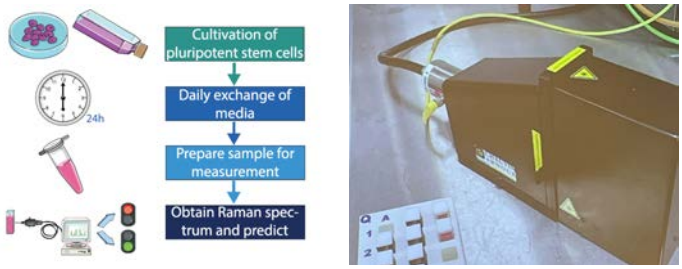


Figure 1:
Experimental setup for determining stem cell pluripotency by Raman spectroscopy

Table 1:
Confusion matrix for the SVM-DA model

		Predicted	
		Bad	Good
Actual (CV)	Bad	30	7
	Good	8	66
Actual (Val)	Bad	11	2
	Good	0	22

CV= Cross-Validation ; Val=Validation

Results and discussion

All the data analyses were performed using MATLAB R2021b and PLS_Toolbox from Eigenvector.

Supervised classification models have been developed (PLS-DA, SVM-DA) to discriminate between samples with good and poor pluripotency.

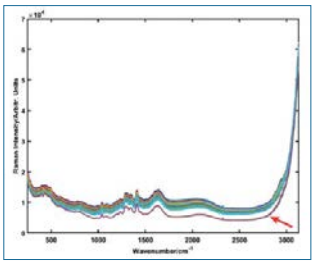


Figure 2:
Raw spectra of N = 153, spent sample medium. The red arrow indicates the outlier spectra. These spectra were deleted before chemometric analysis.

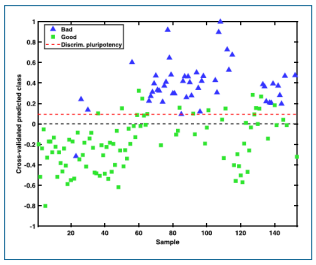


Figure 3:
Prediction of pluripotency by cross-validation with the 8-component PLS-DA model. The samples are colored according to their classes.

The two classification algorithms used in this study are very powerful. The PLS-DA model gives access to robust detection of aberrant values, while the SVM-DA can be used to model the non-linearities within the data, thus improving PSCs pluripotency prediction.

In this study, an at-line analytical technology was developed to determine pluripotency status using Raman spectroscopy and chemometrics.

It was possible to precisely model the non-linear expressions of pluripotency in the extra-cellular matrix. his method reduces analysis time from several hours to 10 minutes, making it possible to increase the information on the status of a cell culture.

Our teams at INDATECH will be delighted to answer your questions by telephone:
+33 4 80 78 01 40

Advantages of the Viserion Raman spectrometer

Raman technology offers a flexible solution for in/at-line testing of cell cultures:

- Quick measurement enabling monitoring of the pluripotent stem cells' status
- A set of probes, flow-cells and vial analyzers allowing reliable, reproducible measurements
- Raman spectroscopy combined with chemometrics, enabling physical and chemical characterization of the product of interest

For further information, please refer to the research paper:
Hagedorn, J., Halloin, C., Skibsted, E., Poulsen, L., & Hedegaard, M. A. (2023). Determination of stem cell pluripotency in spent cultivation media by Raman spectroscopy. Journal of Raman Spectroscopy. https://doi.org/10.1002/jrs.6532

906 211 718 - Ed.2 - 05/2025 - LB - Non-contractual document

FRANCE
Indatech
1900, Boulevard de la Lironde
34980 Montferrier-sur-Lez
Tél : +33 4 80 78 01 40
Fax : +33 4 80 78 01 42
sales@indatech.eu
www.indatech.eu

CHAUVIN ARNOUX
INDATECH