Streamlining Bacterial Infection Diagnosis:
Rapid Gram Classification Using FTIR Spectroscopy

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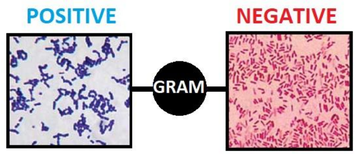
(SOME OF) THE PROBLEM(S)



Gram classification is of the utmost importance in hospitals, in order to properly diagnose bacterial infections and determine the appropriate treatment [1].

However, correctly identifying the Gram classification of a bacterial infection can be challenging due to potential misinterpretation of staining results, overlapping cell morphology, and emerging antibiotic-resistant bacteria [2].

- Therefore, accurate and timely Gram classification is crucial for guiding appropriate antibiotic therapy, preventing infection spread and to help minimize antibiotic resistance [3].
- These challenges only serve to highlight the importance of rapid, economic, and reliable diagnostic methods in a clinic or hospital environment, a critical step to effectively treat and prevent serious complications and life-threatening events for the patients.
- Spectroscopy, especially FTIR spectroscopy present itself as a technique that can enable this goal, as it is can acquire the metabolic status of the biological system in a high sensitivity and specificity mode [4].

AIMS

Evaluate if FTIR spectroscopic analysis of human serum would enable the prediction and correct gram classification in critically ill patients in an ICU environment.

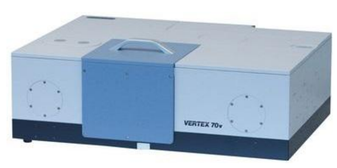
MATERIALS AND METHODS

A. BIOLOGICAL ASSAY: 29 ICU patients with bacteraemia

24 ♂ 5 ♀ → Taking of blood samples → All patients were submitted to blood microbiological analysis, of which the 29 presented bacteraemia. → Waiting for blood to clot → Serum was obtained by **centrifugation** of fresh blood samples. **3000 rpm for 10 min**.

B. MIR SPECTRA ACQUISITION:

Triplicates of 25µl of serum (DF 10) → Transferred to a **96-wells Si plate** → Dehydrated for 2.5h, in a desiccator under vacuum → Spectral data collected with a FTIR spectrometer (Vertex 70, Bruker), equipped with a HTS-XT (Bruker) accessory.



C. SPECTRA PRE-PROCESSING AND PROCESSING:

- **Atmospheric** correction
- **Baseline** correction
- **Unit vector** normalization
- **Second derivative** (2nd order polynomial -15 point window)

→ **t-SNE** (t-distributed stochastic neighbour embedding), performed with: Orange 3.19.0 (*University of Ljubjana, Slovenia*) → **Support Vector Machine (SVM)** learner models were used and a **paired *t*-test** was performed at 1% significance level, to evaluate possible differences in the selected absorbance ratios.

RESULTS

- A total of 29 patients, all hospitalised at an ICU, were considered, all having presented bacteraemia, as based on microbiological analysis. Patients between the two groups (gram positive and gram negative) did not present significant differences concerning gender, age and body mass index ($p > 0.1$).

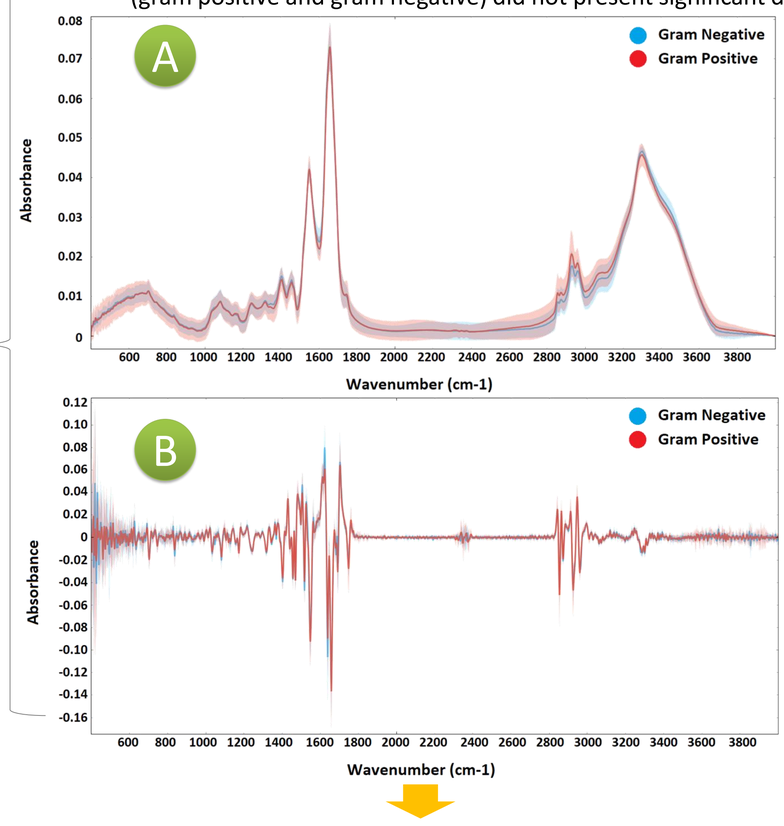


Fig.1. FTIR pre-processed spectra with atmospheric and baseline correction with unit vector normalization (**A**) or normalised second derivative spectra (**B**). In blue, the spectra of the gram negative group and in red the spectra of gram positive group, with shades of the colours representing minimum and maximum values, and solid lines representing their averaged values.

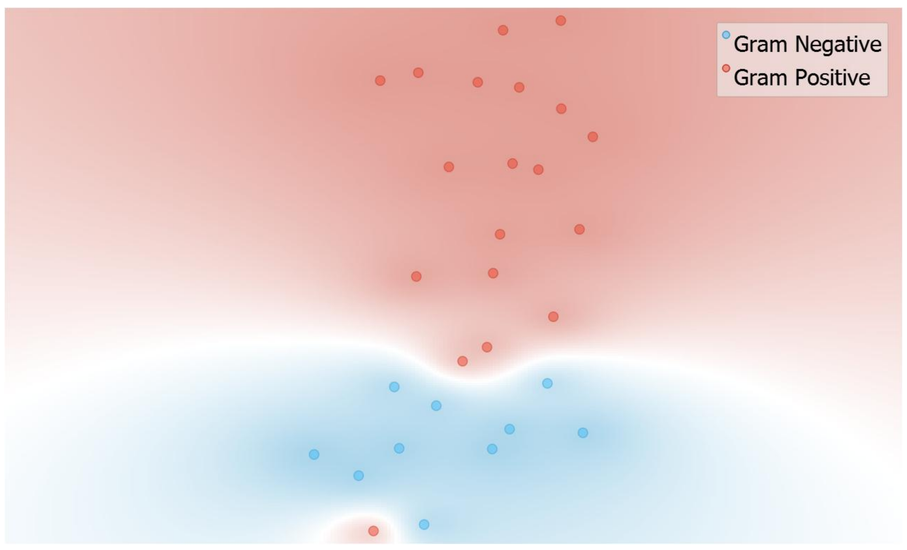


Fig.2. t-SNE of patients with gram negative (blue) and gram positive (red) bacteria, based on serum spectra after a normalized second derivative between 1040-1045 cm-1, 1345-1350 cm-1, 2190 to 2195 cm-1 and 2965-2970 cm-1. The corresponding SVM model can be seen on Table 1 (**D**).

	AUC	Accuracy	Precision	Sensitivity	Specificity
A	0.487	0.643	0.460	0.643	0.324
B	0.460	0.662	0.443	0.662	0.331
C	0.950	0.892	0.892	0.892	0.868
D	**0.969**	**0.897**	**0.898**	**0.897**	**0.883**
E	0.906	0.797	0.797	0.797	0.658
F	0.904	0.880	0.879	0.880	0.815

Table 1. SVM models' performance to predict Gram classification on spectra from serum of 29 patients with atmospheric and baseline correction (**A**), complete spectra normalized second derivative (**B**), and select sub-regions (**C**), normalized second derivative between 1040-1045 cm^{-1}, 1345-1350 cm^{-1}, 2190 to 2195 cm^{-1} and 2965-2970 cm^{-1} (**D**), and normalized second derivative between 600-1800 and 2800-3100 cm^{-1} (**E**) as well as select sub-regions (**F**).

CONCLUSIONS

The present work points to an alternative technique to classify, using FTIR spectroscopy, between positive and negative Gram bacteria in the blood stream, through a simple, rapid, and economic mode, in ICU patients. This allows adequate treatment to critically ill patients, leading to fast infection detection rates, and individually concocted course of antibiotics, at the same time avoid its unnecessary use, which ultimately would ease the metabolic burden on the patients.

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