aRPa FVG
agenzia regionale per la protezione dell'ambiente del Friuli Venezia Giulia

Sistema Nazionale per la Protezione dell'Ambiente

UNIVERSITÀ DEGLI STUDI DI UDINE
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ARPA FVG LABORATORY EXPERIENCE

EVALUATION OF 2D 3D PLOTS AND COMPARISON WITH LATEX AGGLUTINATION TEST FOR *LEGIONELLA PNEUMOPHILA*

BRUKER'S FOURIER TRANSFORM-INFRARED (FT-IR) IR BIOTYPER®

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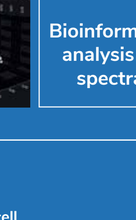
INTRODUCTION

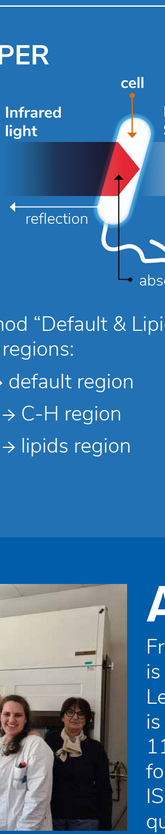
83 isolates of ***Legionella pneumophila*** from water samples, from 2005 to 2017, stored at -80°C, were identified by **MALDI-TOF** and then **sequenced in WGS**, which identified **8 cluster** based on genetic characteristics of the strains, identified with different colors.

The strains were serotyped by:

LATEX AGGLUTINATION TEST
(Oxoid, Thermo Fisher Diagnostic Reagent)

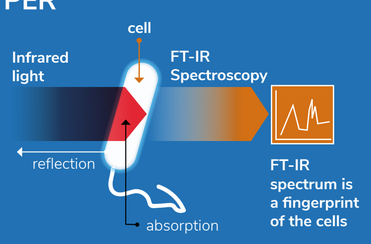


BRUKER'S FOURIER TRANSFORM-INFRARED (FT-IR), IR BIOTYPER ®



FT-IR BIOTYPER

IR spectroscopy is based on the absorption of infrared light by the substance to be measured



The splicing method "Default & Lipids" considers three different IR regions:

1300-800 cm^{-1} → default region

3000-2800 cm^{-1} → C-H region

1500-1400 cm^{-1} → lipids region

AIMS

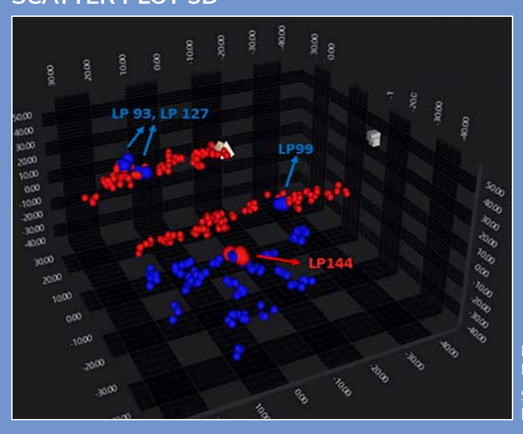
COMPARISON OF SEROTYPING BY LATEX AGGLUTINATION TEST AND FT-IR

STUDY OF STRAINS DISTRIBUTION IN 3D SCATTER PLOT, IN RELATION TO CLUSTERS IDENTIFIED IN WGS

STUDY OF STRAINS DISTRIBUTION IN 2D AND 3D SCATTER PLOT IN RELATION TO THE PRESENCE OF ANTIBIOTIC RESISTANCE GENES (LpeA, LpeB, APH (9)-Ia)

RESULTS

SCATTER PLOT 3D



Exploration method: LDA (0,95-20)
Splicing method: Default & Lipids

SG 1 and SG 2-15 clusters are clearly separated: SG 1 red and SG 2-15 blue.

We identified two groups of SG 1, one of them aligned with L.pn. SG 1 ATCC reference strain.

We found 4/84 (4,8 %) miss-classification latex–FT-IR and we confirmed FT-IR results after the re-execution of latex test.

SCATTER PLOT 3D

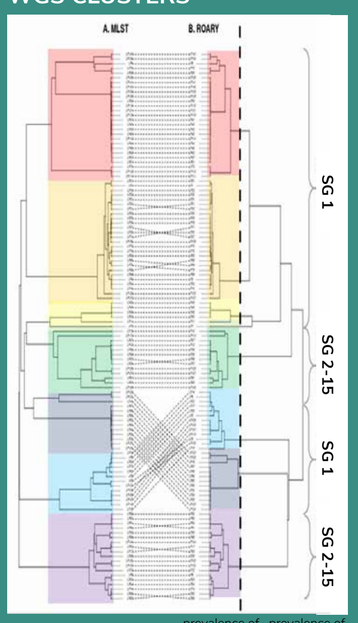
We identified two groups of SG1, one of them (the pink one) aligned with L.pn. SG 1 ATCC reference strain, and another (yellow) separated, as identify by WGS.

The third group includes the other strains, in prevalence SG 2-15.

Exploration method: LDA (0,95-20)
Splicing method: Default & Lipids

Legionella pneumophila SG 1
Legionella pneumophila SG 1
Negative control: *Salmonella enterica* ATCC
Positive control: *Legionella pneumophila* SG1 ATCC

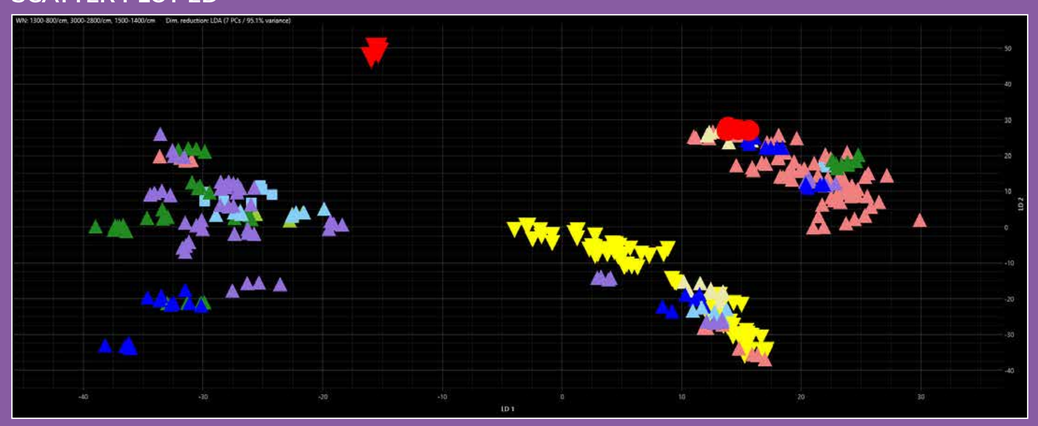
WGS CLUSTERS



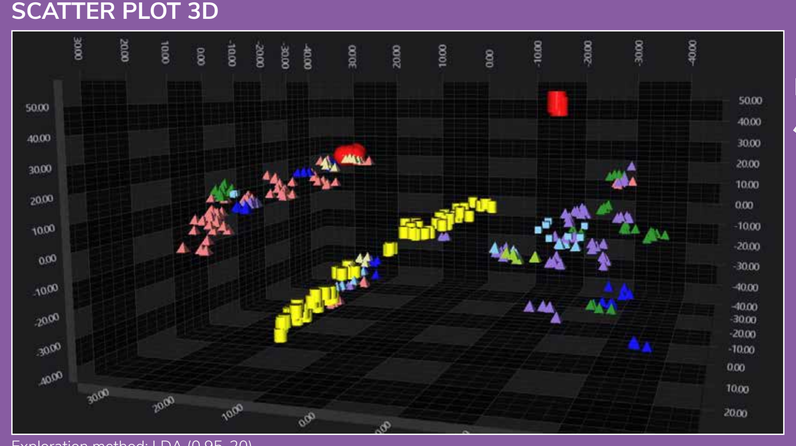
WGS identified **8 cluster** based on genetic characteristics of the strains, identified with different colors.

prevalence of **SG 1**
prevalence of **SG 2-15**

SCATTER PLOT 2D



SCATTER PLOT 3D



Exploration method: LDA (0,95-20)
Splicing method: Default & Lipids

The yellow group of SG 1 showed a prevalence of LpeA and LpeB genes' presence.

The pink SG 1 group, aligned with L.pn. SG 1 ATCC reference strain, showed a clear prevalence of non-positive strains for target genes.

DISCUSSION

FT-IR technology can be used as a valid method for L. pn serotyping.

Clusters identified in WGS correspond to the groups identified in FT-IR, except when other genetic characteristics of the isolate prevail over those that determine its serogroups.

Strains from the yellow cluster come from elderlies, hospitals and community homes, with a greater development of antibiotic resistance, compared to the pink strains, which come from private homes and hotels.

ARPA FVG

From 2002, ARPA FVG Udine Laboratory is the reference centre for Environmental Legionellosis Diagnosis. The laboratory is accredited ISO 17025:2017, ISO 11731:2017 for cultural method for the isolation of Legionella and ISO 12869:2019 for detection and quantification of Legionella using qPCR.

CONCLUSIONS

Results confirmed 100 % correspondence between latex and FT-IR method, showing that FT-IR technology can be used as a valid method for L.pn. serotyping, underlining the possibility to find erroneous latex serotyping.

Furthermore, WGS sequencing can be a valuable support in order to understand if there are other characteristics that determine the spatial distribution of isolates in different clusters.