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COULD MALDI-TOF REPRESENT AN ALTERNATIVE TO SALMONELLA CONVENTIONAL SEROTYPING?

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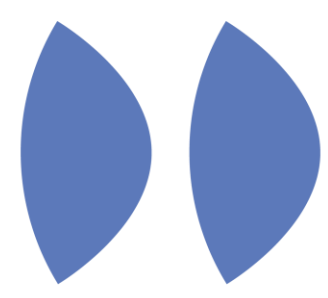
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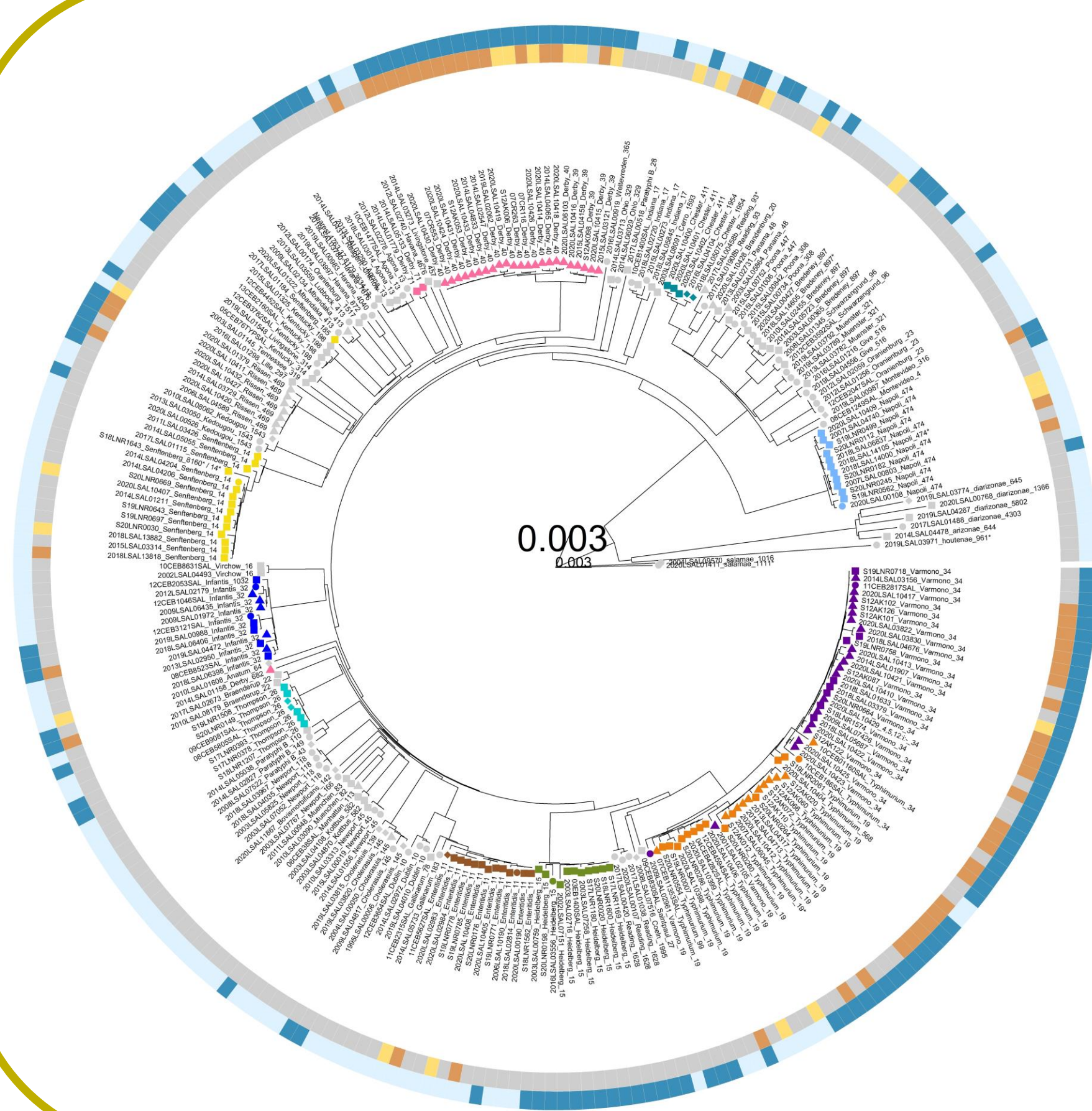
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The identification of the *Salmonella* serotypes obtained by slide-agglutination tests following the Kauffmann-White-Le Minor system¹ is still today considered as the reference method (FD CEN ISO 6579-3). Nevertheless, this method requires a large panel of antisera, it is time consuming and expensive. Laboratories implied in surveillance need a rapid and not-expensive identification method for the most frequently isolated *Salmonella* serotypes. MALDI-TOF/MS could represent an alternative, however even if its ability to discriminate pathogens at genus and specie level was well established, at serotype level it remains scarce. We explore in this study the process to create a good quality *Salmonella* database at serotype level. The ultimate goal being to evaluate the potential of MALDI-TOF for identifying the 10 serotypes considered to be of public health significance.

Step 1: Chose well characterized *Salmonella* strains



Serotype

- Chester
- Derby
- Enteritidis
- Heidelberg
- Infantis
- Napoli
- Other
- Senftenberg
- Thompson
- Typhimurium
- S. 4,[5],12:-

Origin

- Other
- Pork
- Poultry
- Unknown

ATB Heatmap

- Resistant phenotype
- Non resistant
- Unrealized
- ATB genes detected
- no ATB genes detected

- 272 *Salmonella* French strains, including 138 belonging to the 10 targeted serotypes, were phenotypically and genotypically characterized. These strains were from 3 collections of the Anses located at:
 - Food safety laboratory in Maisons Alfort (*Salmonella* Network),
 - Ploufragan-Plouzané-Niort Laboratory in Ploufragan (National Reference Laboratory (NRL) for *Salmonella*),
 - Fougères Laboratory (NRL for antimicrobial resistance).
- All strains were identified by glass slide agglutination, according to the White-Kauffmann-Le Minor scheme¹. For all strains from the NRL for antimicrobial resistance, antibiotic susceptibility was determined using the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI).
- The serotypes were verified genomically by SISTR² prediction and the MLST profile determined by MLSTseeman tool³.
- The accessory genome was explored for resistance genes with ABRicate workflow⁴ and ResFinder, BactMet and vfdb databases^{5, 6, 7}.
- A pan genome phylogenetic analysis was carried out by kmer approach using QuickPhylo tool.

Figure 1. In the tree, the 10 major serotypes are highlighted by coloured dots. The ID of the strains and their MLST profiles are reported. The origin of each strains are indicated by geometric symbols as described in the legend to the right of the tree. The presence or absence of antibiotic resistance genes appears in the ring as well as phenotypical susceptibility (when available).

Step 2: Standardize the analytical process for MALDI-Tof spectra acquisition

- For each of the 10 major serotypes, between 2 to 10 strains were selected based on their genomic diversity and divided into two groups. One to create the reference spectra and the other to validate them. All these strains were analysed with a Microflex-LT after a full proteomic extraction.
- The HCCA (α -Cyano-4-hydroxycinnamic acid) matrix was used and 8 deposits were run 4 independent times to acquire 32 spectra for each strain.
- The reference spectra were produced for the 10 major serotypes with 58 strains after have grown each strain on 2 different culture media (Drigalski and TSYE) in 3 separate series.
- More than 2 000 spectra have been produced for the 10 major serotypes of interest and 30 strains used for evaluating accuracy of database creation. The spectra were cleaned by in-house tool (QualiBioMaldi-Tof).
- The repeatability and reproducibility were evaluated using different statistical and graphical approaches such as Friedman, score analysis, Gelview spectra alignment and ClinProTools methods.

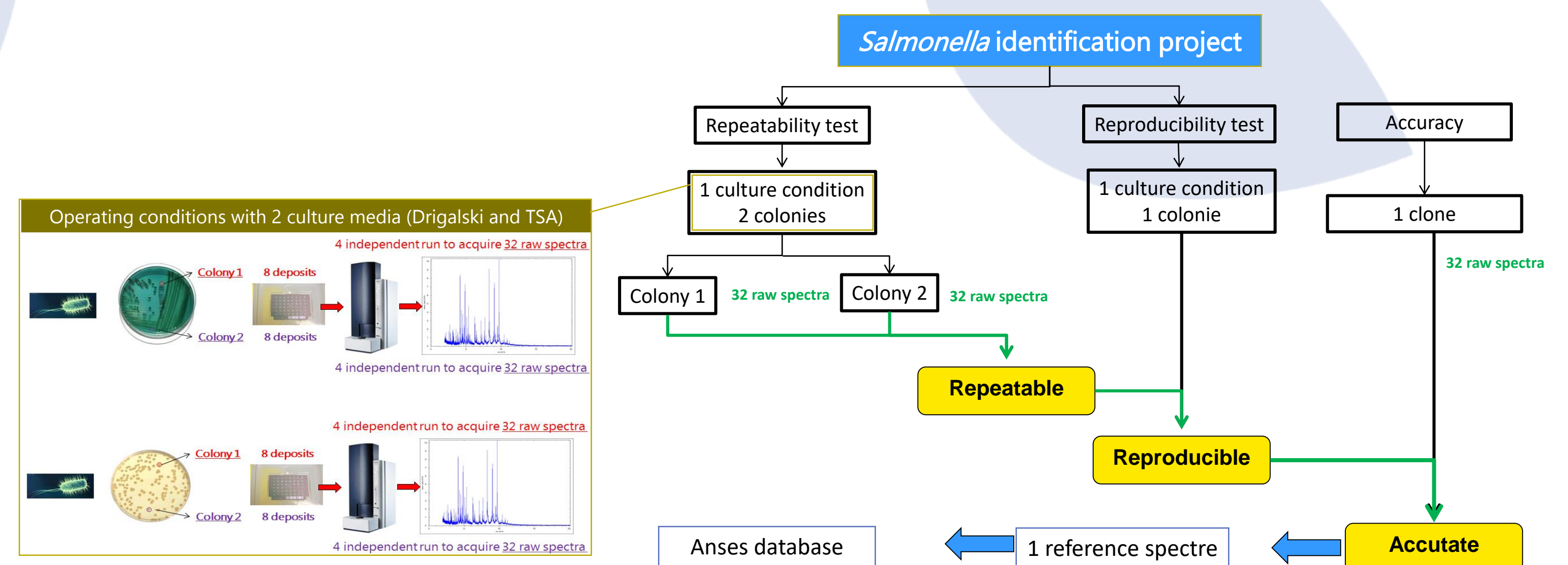


Figure 2. Scheme illustrating the approach used for the creation of spectra and the validation process testing the accuracy of database creation.

Step 3: Standardize the statistical approach for creation of MALDI-Tof database

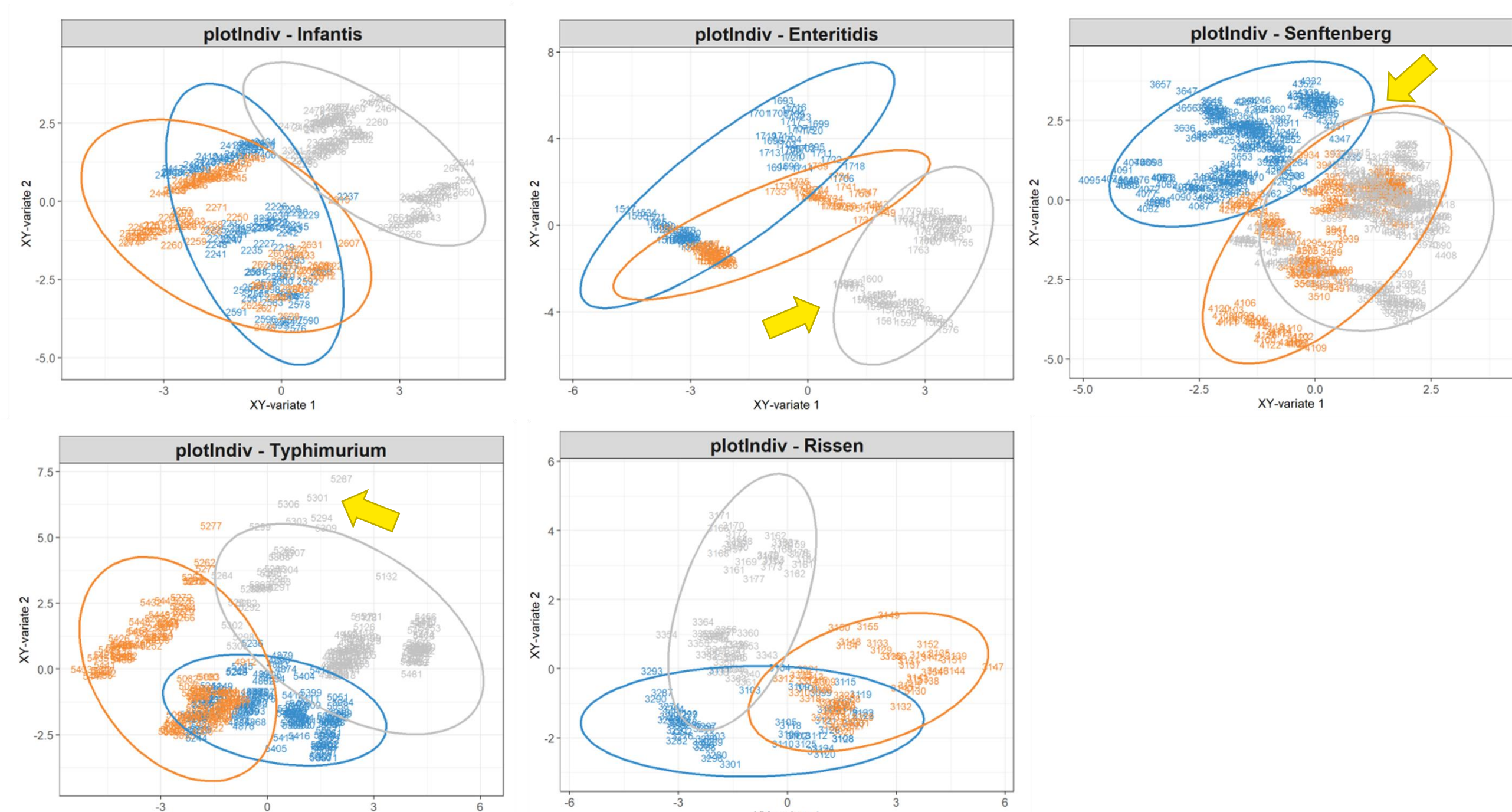


Figure 3. Comparison of statistical approaches for the 3 measures in TSYE medium by PLS-DA sparse analysis for serotypes Infantis, Enteritidis, Typhimurium, Rissen and Senftenberg. Legend: 1 and 2 correspond to repeatability measures and 3 to reproducibility measure. Yellow arrows indicate some major results described in the text.

- Comparisons of the results obtained using different statistical and graphical approaches for 3 measures (2 repeatability and 1 reproducibility measures) and 2 cultures media was carried out by Sparse Partial Least Square discriminant analysis (PLS-DA sparse). Results obtained allowed us to underline that evaluation of the reproducibility was critical step which should be explored prior creation of a database at serotype level.
- Among the 10 major *Salmonella* serotypes analysed we selected 5 graphics, *S. Infantis*, *S. Enteritidis*, *S. Typhimurium*, *S. Rissen* and *S. Senftenberg* to illustrate the data dispersion between reference and validation spectra. For example, for *S. Enteritidis* we can observe that measures for one strain are different. Contrary, for *S. Seftenberg* measures between strains are less dispersed.
- *Salmonella* Typhimurium graphic illustrates well that for a better identification quality, it is important to do at least 5 deposits run one independent time for each strain.

➡ Our results suggests that to create a reference spectra database at serotype level by MALDI-TOF/MS, an integrative approach combining both well characterised strains and different culture media must be chosen. Moreover different statistical approaches to analyse spectra are need.

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