

MICROBIAL CLASSIFICATION PERFORMANCE BY THE SCANSTATION SOFTWARE V9

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Unit: ScanStation® - Software version: 9

Introduction

Manufacturing companies, such as Pharmaceutical companies, are always incorporating new technologies in their workflow to assure the successful execution of their continuous production processes. The performance of Environmental Monitoring (EM) is key to understand the conditions of each production area. Furthermore, to have information in real-time about the type of microorganism(s) detected is extremely important for the design of the disinfection strategy. The ScanStation, real-time incubator and colony counter, is a powerful tool not only to count colonies in real-time (1) but also to classify them as bacteria or yeasts/molds. The new ScanStation software V9 includes an Ai neural network trained on identifying molds based on their phenotypes. Initially, all colonies are detected and identified as bacteria. But, with the progress of the incubation, the Ai neural network classifies them as yeasts/molds if criteria, such as colony morphology, are met.

The ScanStation Software V9 has underwent several upgrades to improve its capability of classifying microbes as bacteria or yeasts/molds. The aim of this study is to evaluate the improvement of the performance of the ScanStation Software V9 to classify microbes as bacteria or yeasts/ molds. A comparison between the classification performed by the ScanStation Software V9 and Manual Classification (Reference Method) is also offered.

Protocol

This study was carried out with bacteria, yeasts and molds and more than 100 strains thereof

where tested (Table #1). Pure cultures of each strain (Biomérieux BioBall) were plated on TSA/SDA agar plates. The optimal temperature was 32.5 °C for bacteria and 22.5 °C for yeasts and molds. Samples were prepared in duplicate. Incubation was run with ScanStation Software V9 DLL V4.01/ **MoBIA 1.0** and ScanStation Software V9 DLL V4.0.10/ **MoBIA 1.3**, respectively. **Bacteria** were labeled with a **cross (+)** while **yeasts/molds** were labeled with a **star (★)**. The classification of bacteria and yeasts/ molds between both versions of the software were compared.

Table #1. Pure cultures of microbes (bacteria- green, yeasts/molds- blue-) used to test the improvement of the ScanStation Software to classify microorganisms as bacteria or yeasts/molds.

Strain
<i>Acaulium acremonium</i>
<i>Alternaria alternata</i>
<i>Aspergillus brasiliensis</i>
<i>Aspergillus caesiellus</i>
<i>Aspergillus flavus</i>
<i>Aspergillus fumigatus</i>
<i>Aspergillus oryzae</i>
<i>Aspergillus terreus</i>
<i>Aspergillus versicolor</i>
<i>Bjerkandera adusta</i>
<i>Botrytis cinera</i>
<i>Chaetomium globosum</i>
<i>Chrysosporium keratinophilum</i>
<i>Cladosporium cladosporioides</i>
<i>Cladosporium herbarum</i>
<i>Cladosporium spp</i>
<i>Curvularia aeria</i>
<i>Curvularia hominis</i>
<i>Curvularia lunata</i>

<i>Curvularia pallescens</i>
<i>Curvularia verruculosa</i>
<i>Eurotium rubrum</i>
<i>Fusarium acuminatum</i>
<i>Fusarium oxysporum</i>
<i>Fusarium proliferatum</i>
<i>Fusarium solani</i>
<i>Ganoderma resinaceum</i>
<i>Helicomyces roseus</i>
<i>Hormoconis resinae</i>
<i>Leptosphaeria maculans</i>
<i>Mucor circinelloides</i>
<i>Mucor hiemalis</i>
<i>Mucor racemosus</i>
<i>Myrothecium verrucaria</i>
<i>Neosartorya fischeri</i>
<i>Nigrospora sphaerica</i>
<i>Paecilomyces variotii</i>
<i>Penicillium aurantiogriseum hirsutum</i>
<i>Penicillium aurantiogriseum</i> var. <i>aurantiogriseum</i> MUCL 29184
<i>Penicillium chrysogenum</i>
<i>Penicillium citrinum</i>
<i>Penicillium expansum</i>
<i>Penicillium italicum</i>
<i>Penicillium rubens</i>
<i>Penicillium venetum</i>
<i>Phialophora</i> spp
<i>Phoma</i> spp <i>herbarum</i>
<i>Purpureocillium lilacinum</i>
<i>Rhizomucor pusillus</i>
<i>Sarocladium strictum</i>
<i>Scopulariosi brevicaulis</i>
<i>Trichoderma citrinoviride</i>
<i>Trichoderma harzianum</i>
<i>Candida albicans</i>
<i>Candida tropicalis</i>
<i>Trichophyton mentagrophytes interdigitale</i>
<i>Acinetobacter baumannii</i>
<i>Aeromonas hydrophila</i>
<i>Alcaligenes faecalis</i>
<i>Bacillus cereus</i>
<i>Bacillus licheniformis</i>
<i>Burkholderia cepacia</i>
<i>Burkholderia gladioli</i>
<i>Citrobacter braakii</i>
<i>Corynebacterium striatum</i>
<i>Cryptococcus neoformans</i>

<i>Debaryomyces hansenii</i>
<i>Debaryomyces maramus</i>
<i>Edwardsiella tarda</i>
<i>Enterobacter cloacae</i>
<i>Enterococcus faecium</i>
<i>Escherichia coli</i>
<i>Escherichia hermanii</i>
<i>Hafnia alvei</i>
<i>Klebsiella aerogenes</i>
<i>Klebsiella oxytoca</i>
<i>Klebsiella pneumoniae</i>
<i>Kocuria rhizophila</i>
<i>Lactobacillus casei</i>
<i>Lactococcus lactis</i>
<i>Latilactobacillus sakei</i>
<i>Listeria monocytogenes</i>
<i>Micrococcus luteus</i>
<i>Morganella morgani</i>
<i>Myroides odoratus</i>
<i>Pantoea agglomerans</i>
<i>Papiliotrema laurentii</i>
<i>Proteus mirabilis</i>
<i>Pseudoescherichia vulneris</i>
<i>Pseudomonas aeruginosa</i>
<i>Pseudomonas fluorescens</i>
<i>Pseudomonas stutzeri</i>
<i>Rahnella aquatilis</i>
<i>Rhodococcus equi</i>
<i>Rhodotorula glutinis</i>
<i>Rhodotorula mucilaginosa</i>
<i>Saccharomyces cerevisiae</i>
<i>Salmonella typhimurium</i>
<i>Shigella flexneri</i>
<i>Staphylococcus aureus</i>
<i>Staphylococcus epidermidis</i>
<i>Streptococcus pyogenes</i>
<i>Yarrowia lipolytica</i>

To validate this study, the classification performed, respectively, by the ScanStation Software V9 (DLL V4.01/ **MoBIA 1.0**) and by the ScanStation Software V9 (DLL V4.0.10/ **MoBIA 1.3**) were compared with Manual Classification (Reference Method). The correlation coefficient (R^2) was determined.

Results

1.Improvement of the ScanStation Software V9 to classify microorganisms as bacteria and yeasts/ molds, respectively.

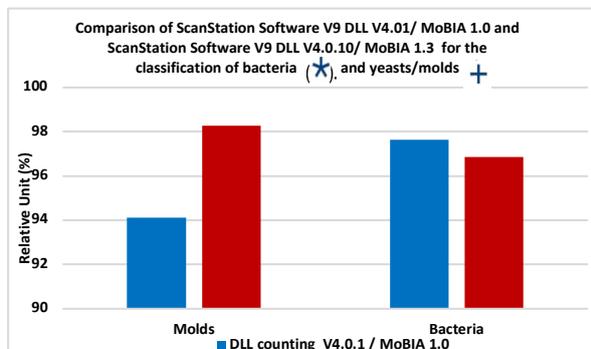


Fig 1. Improvement of ScanStation Software V9 for the classification of microbes in either bacteria + or yeasts/ molds (*).

Fig. 1 shows the classification of bacteria and yeasts/ molds performed by two (2) different versions of the ScanStation Software V9. Results are expressed in percentage (%) and correspond to the bacteria and yeasts/ molds on the samples correctly classified. For the classification of bacteria, no significant difference between version DLL V4.0.1/ MoBIA 1.0 and version DLL V4.0.10/ MoBIA 1.3 was found. The corresponding standard deviation (std) between both versions was 0.560. For the classification of yeasts/ molds, ScanStation Software V9 DLL V4.0.10/ MoBIA 1.3 shows a slightly improvement compared to version DLL V4.0.1/ MoBIA 1.0. The standard deviation (std) between both software was closed to 3. This result can be explained with the improvement in the classification of critical samples as shown in Table #2. The classification of yeasts/ molds increased between approximately 15- 50 %.

Table #2. Critical samples showing a significant improvement in classification by the ScanStation Software V9 DLL V4.0.10/ MoBIA 1.3

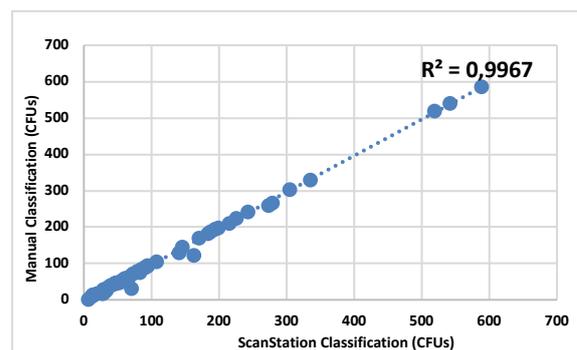
Strain	ScanStation Software V9 DLL V4.0.1 / MoBIA 1.0 (%)	ScanStation Software V9 DLL V4.0.10 / MoBIA 1.3 (%)
<i>Aspergillus fumigatus</i>	86	100
<i>Myrothecium verrucaria</i>	46	91
<i>Paecilomyces variotii</i>	75	100
Penicillium aurantiogriseum var. aurantiogriseum MUCL 29184	63	100

<i>Trichophyton mentagrophytes interdigitale</i>	80	100
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2. Comparison between microorganisms' classification by the ScanStation Software V9 and Manual Classification (Reference Method)

ScanStation Software V9 DLL V4.0.1/ MoBIA 1.0

2.a) Yeasts & Molds



2.b) Bacteria

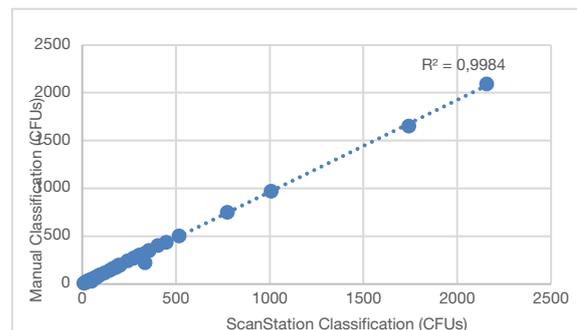
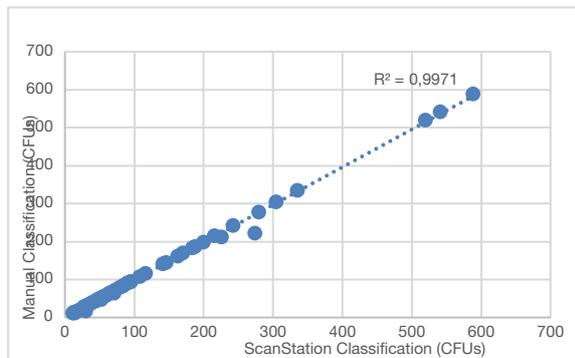


Fig 2. Correlation between Manual Classification (Reference Method) and ScanStation Software V9 DLL V4.0.1/ MoBIA 1.0 classification of yeasts/ molds (2.a) and bacteria (2.b).

Fig. 2 shows the correlation between Manual Classification and ScanStation Software V9 DLL V4.0.1/ MoBIA 1.0 classification of yeasts/ molds (2.a) and bacteria (2.b). The correlation coefficient (R^2) for the classification of, respectively, yeasts/ molds (2.a) and bacteria (2.b) is close to 1. Therefore, there is not significant difference between Manual Classification (Reference Method) and ScanStation Software V9 DLL V4.0.1/ MoBIA 1.0.

ScanStation Software V9 DLL V4.0.10/ MoBIA 1.3

2.c) Yeasts & Molds



2.d) Bacteria

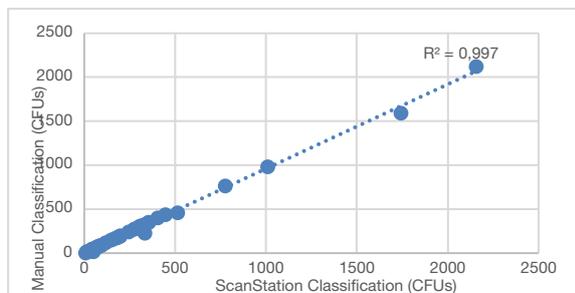


Fig 3. Correlation between Manual Classification (Reference Method) and ScanStation Software V9 DLL V4.0.10/ MoBIA 1.3 classification of yeasts/ molds (2.c) and bacteria (2.d).

Fig. 3 shows the correlation between Manual Classification and ScanStation Software V9 DLL V4.0.10/ MoBIA 1.3 classification of yeasts/ molds (2.c) and bacteria (2.d). The correlation coefficient (R^2) for the classification of, respectively, yeasts/ molds (2.c) and bacteria (2.d) is close to 1. Therefore, there is not significant difference between Manual Classification (Reference Method) and ScanStation Software V9 DLL V4.0.10/ MoBIA 1.3.

Conclusion

The comparison established in this study between ScanStation Software V9 DLL V4.0.1/ MoBIA 1.0 and ScanStation Software V9 DLL V4.0.10/ MoBIA 1.3 shows that both versions of the software are capable of classifying bacteria and yeasts/molds with high precision. However, the classification of yeasts/ molds was improved in the latest version of ScanStation V9 software.

The improvement of the Ai neural network to classify microbes is always an *on- going process* which is accomplished with the collaboration of our clients who are constantly testing new strain of microbes to be classified.

References

¹ Sibileau L., Alexandre T. (2022) La Vague N° 74. Real- Time detection of CFU growth with the ScanStation smart incubator expedites the environmental monitoring process, 37- 43.