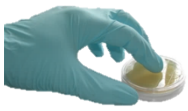
SANOFI PASTEUR

interscience
pour la microbiologie



Performance results obtained with ScanStation, an automate for plate incubation, colony detection and enumeration

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Initial situation prior to the initiative

Microbial testing in the pharmaceutical industry serves a critical role in product safety. In the case of environmental monitoring (EM), microbial testing of air, surfaces and personnel is constantly performed to confirm that microbial contamination is maintained within defined acceptable limits. The current environmental monitoring requirements are highly constraining in production: number of tests, lead time, holding time, resources, and costs.

In product cycle time, QC activities represent around 70% and are often considered as bottle necks. The request for microbiological EM testing is hugely increasing the short further years, due to regulatory requirements. This situation is an opportunity to evaluate automation technologies for microbial plate incubation and reading.

→ The objective of our collaborative study within Interscience is to generate new results from local microflora and to evaluate some performance criterias colony detection on different agar plates (supplier, media composition or format), time-to-results, and comparison between operator and automate for enumeration.

ScanStation SPECIFICATIONS

… a real-time colony counting station, performing fully automatic incubation, detection and enumeration on the Agar plates.

- High resolution color images
- Kinetic reading every 30 minutes
- Specific algorithm to analyze images
  - Delivering a video recording of the microbial growth
- Incubator with regulated temperature (± 0.5 C°) and a capacity of 100 Petri dishes
  - Compatible with the usual plates (55 & 90 mm) and contact plates
  - Incubation at 16-45°C → possibility to program a temperature shift during incubation period
- Automated export of data report (PDF or .CSV format)

The ability to "go back in time" can help validate the results because the operator can review early images to determine the original number of colonies (pointed by a green cross +): → "double-checked" test.

Agar plates for environmental monitoring

- TSA, TSA3P, TS3N, CT 3P
- SDA 3P
- R2A (water testing)

Media for food & cosmetics

- PCA, VRBL, MRS …

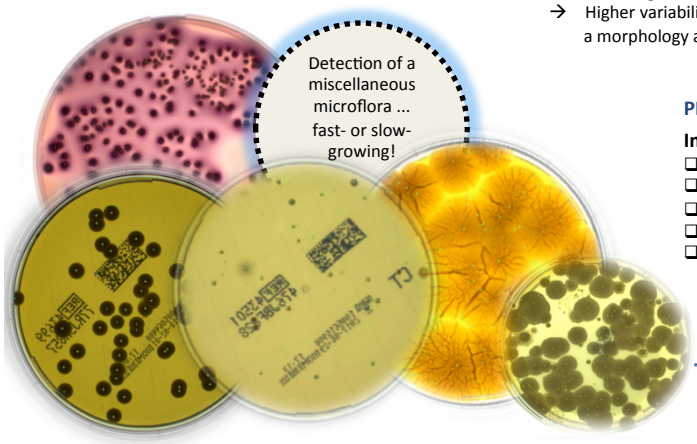
List of micro-organisms tested:

Bacteria species

- **Aeromonas:** *A. hydrophila*
- **Bacillus:** *B. liqueniformis, B. subtilis*
- **Citrobacter:** *C. braakii, C. freundii*
- **Enterobacter:** *E. aerogenes, E. agglomerans, E. cloacae*
- **Enterococcus:** *E. faecalis, E. faecium*
- **Escherichia:** *E. coli*
- **Klebsiella:** *K. pneumoniae*
- **Lactobacillus:** *L. casei* (see picture) →
- **Listeria:** *L. innocua, L. monocytogenes*
- **Methylobacterium:** *M. extorquens*
- **Micrococcus:** *M. luteus*
- **Proteus:** *P. mirabilis, P. vulgaris*
- **Pseudomonas:** *P. aeruginosa ,P. fluorescens*
- **Salmonella:** *S. enteritidis, S. typhimurium*
- **Serratia:** *S. liquefaciens, S. marcescens*
- **Shewanella:** *S. putrefaciens*
- **Staphylococcus:** *S. aureus, S. epidermidis, S. saprophyticus*

Yeast and molds

- **Aspergillus:** *A. brasiliensis, Neosartorya* spp
- **Penicillium:** *P. roqueforti*
- **Candida:** *C. albicans*

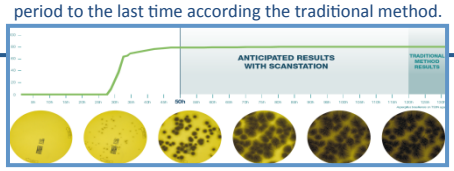


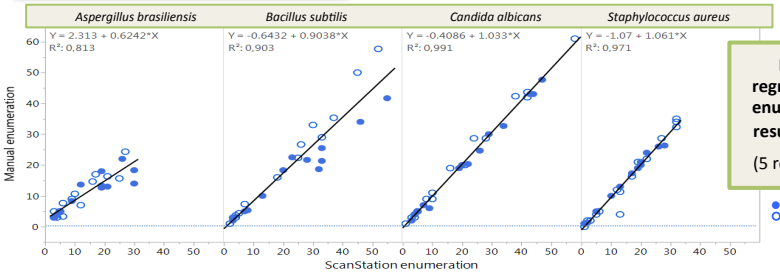
Potent reduction of the lead time … by an early detection:

Microbial species	Colony detection / Final count on TS3N plate
P. aeruginosa	15 hours / 3 days
B. subtilis	15 hours / 3 days
S. aureus	6 hours / 3 days
C. albicans	15 hours / 3 days
A. brasiliensis	40 hours / 5 days

Kinetic growth and "go back in time" feature

Example of *A. brasiliensis* growth reported by ScanStation at different time of incubation on a TSA plate from the earliest period to the last time according the traditional method.





Linear regression of enumeration results (CFU)

(5 replicates)

→ Very satisfactory results for comparison of automate enumeration and manual counting ; linear regression (R^2>0.9) (TS3N and CT 3P plates)

→ Higher variability seems to appear with *Aspergillus* species that exhibit a morphology and a sporulation complicating the enumeration.

By long incubation time, the colony count could be also a challenge for the operator!

PERSPECTIVES …

Implement ALCOA PLUS (+) data integrity

- ❑ Different user access levels & unique identifiers
- ❑ All data generated electronically - audit trail legible
- ❑ A date/time stamp provided on any actions, report and raw data
- ❑ Reports generated automatically and cannot be modified
- ❑ Automated reading & calculation (CFU or CFU/ml) = accurate results
  - ❑ No possibility to delete data
  - ❑ Sequence of all events reported
  - ❑ Data archived electronically & transferred to LIMS
  - ❑ Data available whenever needed

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