Hygiena® MicroSnap® vs 3M™ Petrifilm™ vs bioMérieux TEMPO® Correlation

Objective

About This Study

The goal of this study was to evaluate the correlation between the quantification results obtained from the commercial methods of 3M™ Petrifilm™, bioMérieux TEMPO®, and Hygiena® MicroSnap®.

Equipment, Supplies and Reagents

Bacterial Strains

Four different standard microorganisms were selected to test for the 4 different microbial indicators offered by the methods: Aerobic Plate Count, *Enterobacteriaceae*, Coliforms, and *E. coli*. The specific microorganisms used are described in the following table.

Test Purpose	Microorganism
Aerobic Plate Count (APC)	*Listeria innocua Seeliger*: ATCC 33091
Enterobacteriaceae	*Salmonella enterica* subsp. *enterica* Typhimurium: ATCC 14028
Coliform	*Citrobacter rodentium*: ATCC 51459
E. coli	*Escherichia coli*: ATCC 25922

Media

- BHI agar plates (Brain Heart Infusion)
- BHI broth
- Sterile Loops
- 50 mL conical tubes
- Incubators (30 °C, 35 °C, 37 t°C)
- BPW (Buffered Peptone Water)
- TSA plates (Tryptic Soy Agar)

Methods

Culture Preparation and Concentration Check

Each strain of microorganism was transferred from stock culture onto BHI agar plates to initiate regrowth. Plates were incubated at 37 °C for 18 - 24 hr. From each plate per microorganism, one (1) colony was removed and transferred into 9 mL BHI broth (this was repeated for multiple colonies on each plate). The broth cultures were incubated at 37 °C for 18 - 24 hours to create pure cultures containing ~1 x 10^9 CFU/mL of each microorganism.

Cultures were checked for appropriate concentration levels by serially diluting each pure culture from 10^{-1} to 10^{-8} by transferring 1 mL into 9 mL BPW broth, followed by plating 100 µl of each dilution onto TSA plates (in duplicate). Plates were incubated at 37 °C for 18 - 24 hours and colonies were counted and recorded to calculate original Log CFU/mL.

Microorganism Cocktail Preparation

For each microorganism cocktail, in triplicate, 5 mL of each pure culture were transferred into a 50 mL conical tube to create 3 separate cocktails containing all 4 organisms (total volume = 20 mL per tube). Each cocktail was serially diluted from 10^{-1} to 10^{-8} (1 x 10^8 to 1 x 10^0 CFU/mL) by transferring 1 mL into 9 mL of BPW broth.

From each cocktail (using triplicate dilutions), use ~1 x 10^1 to ~1 x 10^7 CFU/mL concentrations (6 different concentrations) to create 18 independent samples for testing on the three systems: 3M Petrifilm, bioMérieux TEMPO, and Hygiena MicroSnap.

Enumeration Methods

For all 3 methods used (3M Petrifilm, bioMérieux TEMPO, and Hygiena MicroSnap), testing was performed according to the instructions supplied with each system. Testing for Aerobic Plate Count (APC), *Enterobacteriaceae* (EB), *E. coli* (EC), and Coliforms (CC) were performed for all three systems. A summary of the incubation times and temperatures along with subsequent processing steps are indicated in the figure below.

Enumerative Method		
3M™ Petrifilm™	**bioMérieux TEMPO®**	**Hygiena MicroSnap™**
APC - 24 h @ 37 °C EB - 24 h @ 37 °C EC - 24 h @ 37 °C CC - 48 h @ 37 °C	APC - 22 - 27 h @ 35 °C EB - 22 - 24 h @ 35 °C EC - 22 - 24 h @ 35 °C CC - 22 - 27 h @ 35 °C	TVC - 7 h @ 30 °C EB - 6 h @ 37 °C EC - 6 h @ 37 °C CC - 6 h @ 37 °C
Count after incubation, place EC/CC cards back into incubator for CC reading at 48 h	Transfer 1mL into correct dilution vial (figure for dilution sets)	Transfer to detection swab
	Place in TEMPO filler (3 min)	TVC & EB = Direct read EC & CC = 10 min @ 37 °C
	Place filled cards in incubator	
	Read cards following incubation	

Results & Discussion

Method Comparisons

For all three (3) systems, data was collected as CFU/mL (or converted to CFU/mL from RLUs) and converted to Log CFU/mL for statistical comparison. Statistical significance was observed at alpha 0.05.

At the same time, the work flow for each method was evaluated to identify total time to results. A summary of this work flow timeline is shown below:

Stage of Process (Total number (n) / Method)	**TEMPO** (n = 120)	**MicroSnap** (n = 132)	**Petrifilm** (n = 156)
1) Sample Set Up (Start Processing until Incubation)	90 min	25 min	20 min
a) Touch Points (Opening + Closing of Sample)	3	1	1
2) Technician - Minutes per Sample	1.5	0.38	0.26
3) Incubation Times	22 - 27 h	6 - 7 h	24 - 48 h
a) Incubator Needs	Open Air	Hygiena Digital Incubator	Open Air
4) Results Processing	30 min	30 min	30 min
a) Read Type (Batched or Single)	Batched	Single	Single
b) Software (Mapping Capabilities)	No	Yes	No
Total Time to Results (including processing)	24 - 29 hours	7 - 8 hours	25 - 49 hours

Method Feedback

During the testing, all three systems were evaluated for ease of use and technique logistics. Each system had both positive and negative qualities that were identified during sample analysis.

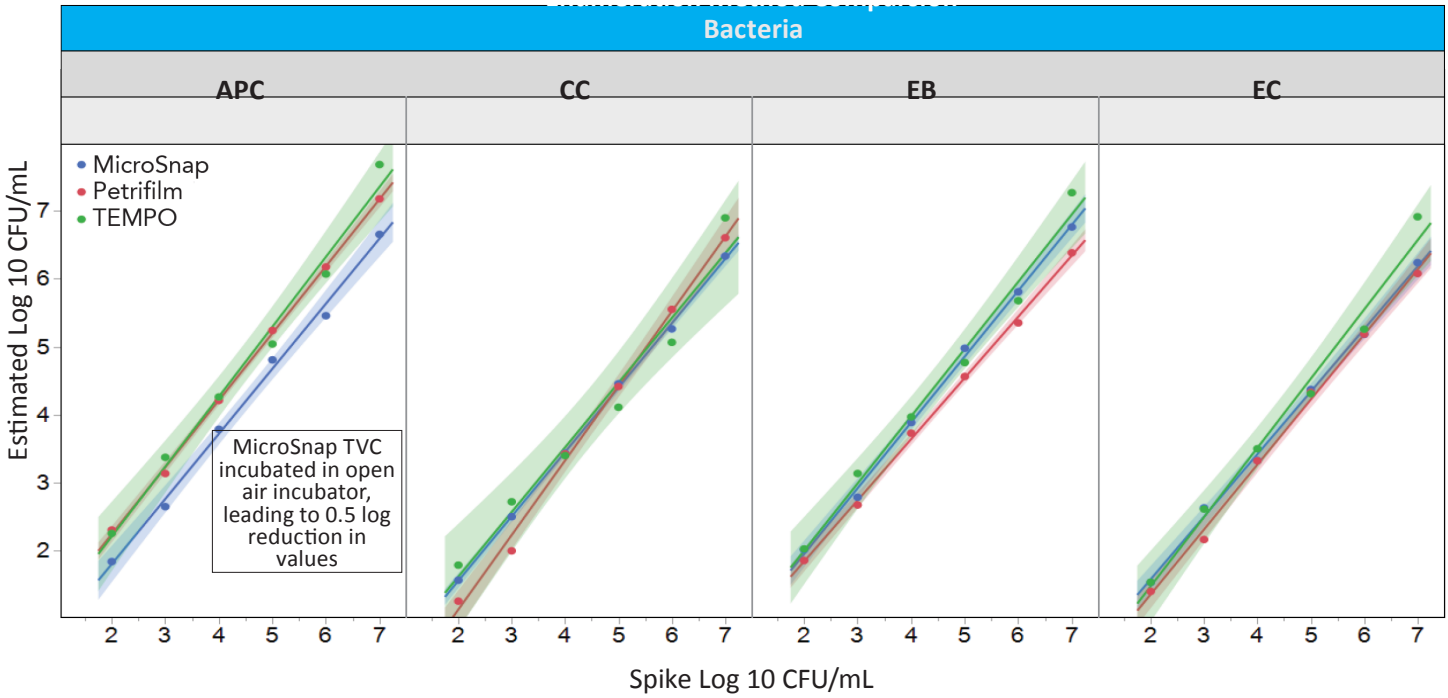
Systems		
TEMPO	**Pro**	**Con**
	Reading results from cards can be done in batches of 20 – quick, not labor intensive	Sample preparation and loading into cards is very labor intensive – must work in batches
		Multiple vial openings slow down operator and can increase error
		Can only label vial – cannot label MPN card (labelled by entering sample ID after scanning barcode)
		Machine locks out sample for specific timepoint
		Requires 22 - 27 hour incubation before reading results
		Card must be read within 45 minutes of pulling from incubator or card is useless
		Must set up baseline of enumerable ranges of sample types

Petrifilm	Pro	Con
	Easy to use	Large area/bench space required to layout films
	Easy to hand write or print labels	Multiple dilutions required to reach countable range and to set up baseline
	Can be pulled from incubator, stored refrigerated until ready to count the same day	Requires 24 - 48 hour incubation before reading results
	Can use automated film counter to determine colony numbers	Additional cost for automated counter and additional space required in lab
	Inexpensive, easy to repeat if plating error occurs	Expensive in terms of labor, time and additional plates/time needed to reach countable range

MicroSnap	Pro	Con
	Easy to use	Requires tube holder/rack for organization
		Bench top incubator required – need larger or multiple incubators for high throughput
	Same day results (6 - 8 hours)	
	Quick read – 10 second read time	Coordination of batching is challenging at first
	Easy to repeat reading using remaining sample in Incubation Device	
	Software for data collection, storage, trending	Aseptic technique a bit challenging – preventing spread of organisms all over equipment

Enumeration Evaluation

With respect to accuracy to detection, all methods were not statistically different in counts for CC, EB, and EC. For APC counts, the MicroSnap devices were placed in an open-air incubator to run parallel with other methods to see what effect this had on results. There was approximately 0.5 Log growth reduction at all levels when the appropriate incubator was not used. TEMPO had a much wider error range when compared to both Petrifilm and MicroSnap across all bacteria counts and microorganism types.



Conclusions

This study demonstrated that all 3 methods (3M Petrifilm, bioMérieux TEMPO, and Hygiena MicroSnap) generate similar CFU counts when each system's procedures are closely followed. If the proper incubator is not used for MicroSnap devices, an approximate growth reduction of 0.5 log is observed (as seen in the APC values above). Nevertheless, the results were in alignment with expected results and comparable to other systems. In addition, the TEMPO method generated wider variability in results across all bacteria counts when compared to Petrifilm or MicroSnap. TEMPO also took longer for sample set up, using valuable operator time. Furthermore, both Petrifilm and TEMPO require an overnight incubation before results can be read while MicroSnap results can be obtained the same day (6 - 8 hours). Overall, MicroSnap is the better option for obtaining results quickly with little operator time required and software for capturing data, eliminating the risk of human error in entering results.

References

1. Petrifilm™ is a trademark of 3M™
2. TEMPO® is a trademark of bioMérieux™
3. MicroSnap™ is a trademark of Hygiena™