RAPID DETECTION OF SPOILAGE MICROORGANISMS IN FRUIT JUICES

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OBJECTIVES AND CONCLUSIONS

The objective is to validate one rapid detection method of spoilage microorganisms, including molds, yeasts, lactic-acid and acetic-acid bacteria in low pH fruit juice ingredients.

The bioMérieux method D-COUNT® 50 based on Flow Cytometry technology, allowed a significant **reduction of time to results from 6 days to 3 days**, providing valid and **equivalent results to the reference cultural method** (International Fruit and Vegetable Juice Association IFU2C).

The **different factors** evaluated in one real industrial laboratory (laboratory technician, enrichment incubation time, sample size and storage enrichment broth) **did not impacted in the performance** characteristics of the method.

MATERIAL AND METHODS

REFERENCE METHOD (IFU2C):
Ten mL of each food item is transferred to 90 mL of sterile Malt Extract Broth and incubated at 30 ºC for 3 days. After enrichment, 1 mL is poured in one Petri Dish with 15-20 mL of Orange Serum Agar and incubated at 30 ºC for another 3 days.

ALTERNATIVE METHOD (D-COUNT® 50 bioMérieux):
From same enrichment 1 mL is taken for analysis consisting in sample preparation (dilution in dispersing buffer, matrix separation & centrifugation), incubation 45 minutes at room temperature and introduced in **automated sample preparation module**. Viable microorganisms are labeled and injected into the **flow cytometer analyser:**

The fluorescence ratio is used to differentiate labeled microorganisms from other particles from the food item. Final results are reported following digital data processing.

FACTORIAL VALIDATION ISO 16140-4:2020:
Twelve food items were tested, including three different types:

- Juice ingredients; juice concentrates and high pigmented ingredients

Four acid-tolerant microorganisms were spiked:

- *Candida sake*
- *Saccharomyces cerevisiae*
- *Torulaspora delbrueckii*
- *Asaia siamensis*

Each food item was analysed in two settings (including four factors at two levels) and three contamination levels (blank, 0.9 and 1,5 cfu/100 mL), producing in total 144 results. In addition, inclusivity (40 strains at 100 cfu) and exclusivity (16 strains at 10,000 cfu) tests were performed with pure cultures. From the set of results, performance characteristics were estimated and compared with acceptability limits from ISO 16140-4:2020.

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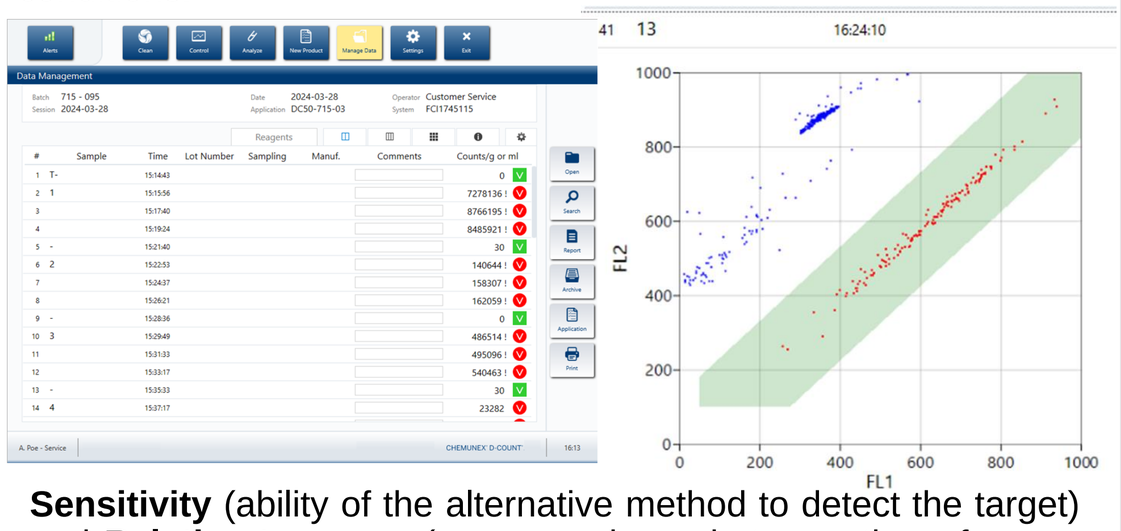
RESULTS AND DISCUSSION

Non spiked samples provided negative results for both methods, showing **no cross contamination** occurred in real industrial conditions.



Sensitivity (ability of the alternative method to detect the target) and **Relative trueness** (correspondence between the reference and the alternative method) was **97.14 %** and **98.16 %** respectively, with no positive deviation (PD) and **no false positives**.
One negative deviation (ND) was detected on sweet cherry juice concentrated spiked with 0.9 cfu/100 mL of *Torulaspora delbrueckii*, where results were positive for the alternative method but below sensitivity limit.

Deviation values (ND and PD) were valid and **below the acceptability limits** for paired studies according ISO 16140-4 (ND-PD=3 and ND+PD=6)

Relative Level of Detection ($RLOD_{50}$) for each food type and for all food types (combined) were below the acceptability limits according ISO 16140-4 for paired study ($RLOD_{50} \leq 1.5$)

Food type	Relative Level of Detection 50%
Juice ingredients	1.000 (CFU/test portion)
Juice concentrates	1.188 (CFU/test portion)
High pigmented ingredients	1.000 (CFU/test portion)
Combined	**1.054 (CFU/test portion)**

Differences for $RLOD_{50}$ between the four factors tested at two levels, were also **within the acceptability limits** included in ISO 16140-4 ($-0.3 < d < 0.3$)

Factor evaluated	Differences $RLOD_{50}$
Lab. technician (A-B)	0.05
Enrichment time (72 h-120 h)	-0.0465
Sample size (10 mL-15 mL)	0,0496
Broth storage (0-2 weeks)	0.0453

Inclusivity showed positive results for all 40 target microorganisms tested. Four negative results for molds became positive after applying a suitable homogenization protocol. Two negative results obtained from vegetative cells from *Alicyclobacillus* were positive when testing spores.

Exclusivity tests from 16 non-target microorganisms where negative after pre-enrichment in acidic conditions.