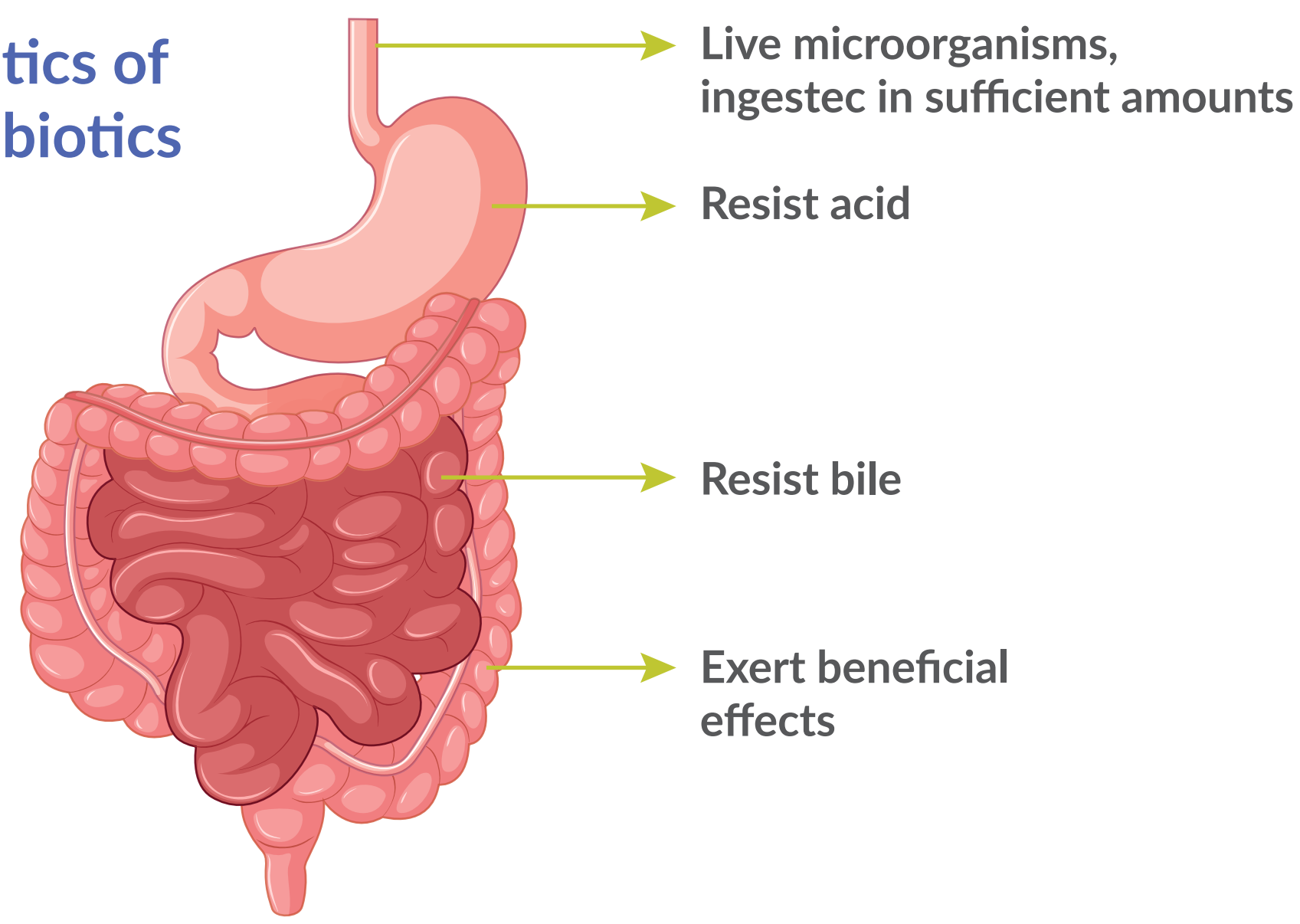


# Compositional Quality and Potential Gastrointestinal Behavior of Probiotics Products Commercialized in Italy

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## INTRODUCTION

### Characteristics of good oral probiotics



This study evaluates quantitative and qualitative aspects and the viability in simulated gastric and intestinal juices of the top 10 commercial probiotic formulations available in Italy.

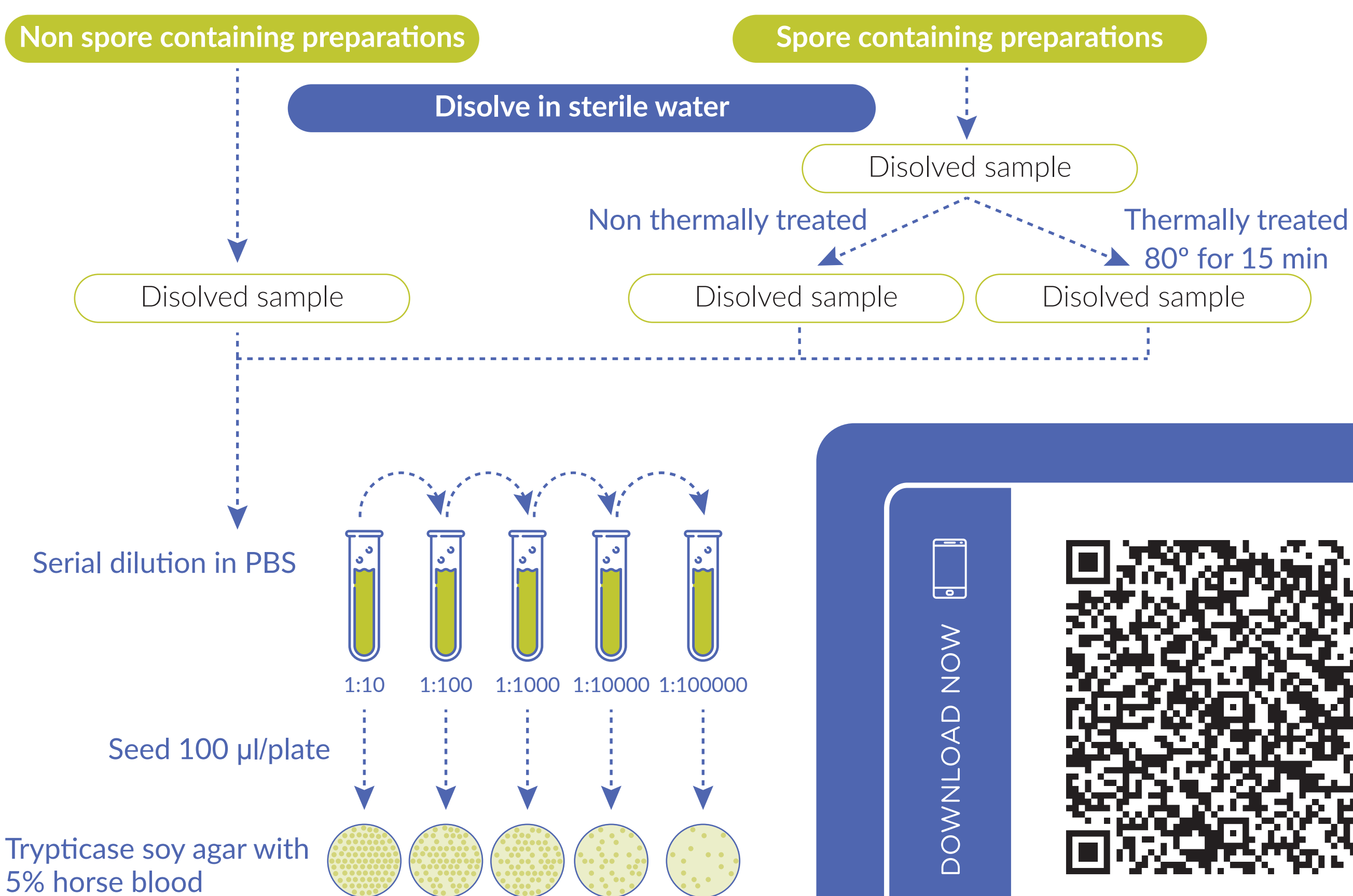
## MATERIALS & METHODS

### Formulations analyzed in this study

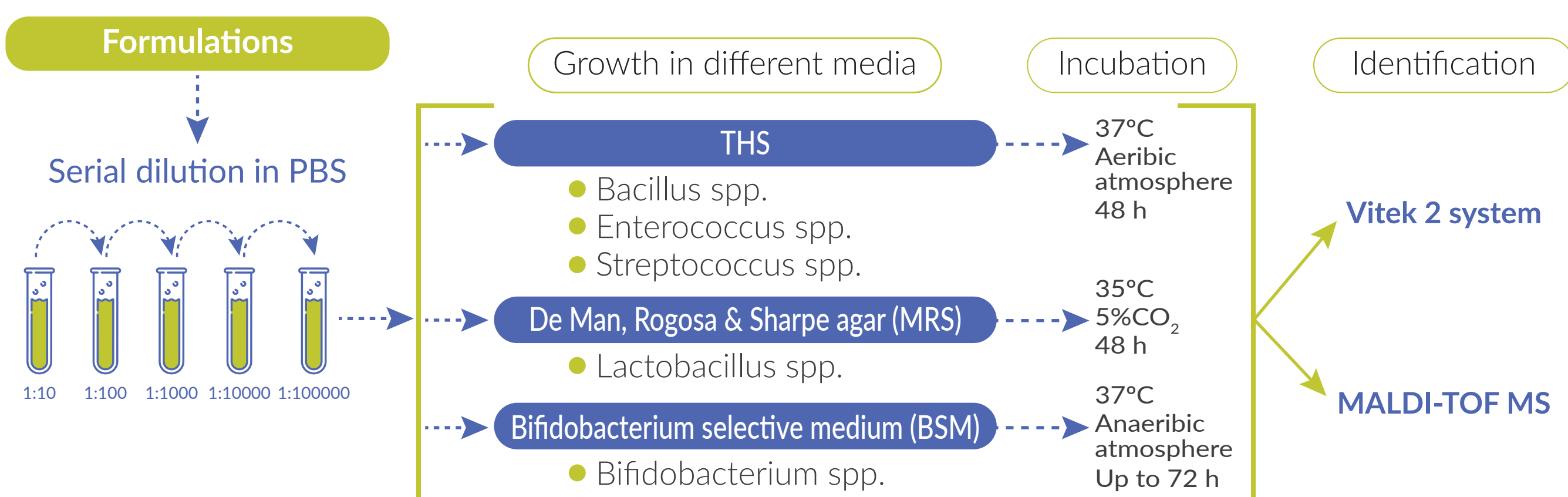
A	B	C	D	E
Bacillus clausii 4 strains S/N, N/R, O/C, T	Lactobacillus paracasei	L. acidophilus L. paracasei Bifidobacterium *B. coagulans	L. reuteri	Saccharomyces boulardii
F	G	H	I	J
*B. coagulans Bifidobacterium lactis Streptococcus thermophilus L. acidophilus L. plantarum L. brevis L. rhamnosus L. casei L. gasseri L. helveticus	L. rhamnosus	S. Cerevisiae sub. boulardii Enterococcus faecium L. acidophilus	S. salivarius subsp. thermophilus B. breve B. infantis B. longum L. acidophilus L. plantarum L. casei L. delbrueckii subsp. bulgaricus	S. thermophilus B. breve B. longum B. infantis L. acidophilus L. plantarum L. delbrueckii subsp. bulgaricus

\* Present as spores

### Microbial enumeration



### Microbial identification



### Microbial viability

Simulated gastric juice	ASTM (American Society of Testing Materials)	<ul style="list-style-type: none"> <li>0.07 N hydrochloric acid</li> <li>pH 1.5</li> <li>37°C</li> <li>Incubated at 37°C for 0, 30, 60, and 120 min</li> </ul>
	USP (U.S. Pharmacopeia):	<ul style="list-style-type: none"> <li>0.03 M sodium chloride</li> <li>0.084 M hydrochloric acid</li> <li>0.32% (w/v) pepsin</li> <li>pH 1.4</li> <li>37°C</li> <li>Incubated at 37°C for 0, 30, 60, and 120 min</li> </ul>
Simulated Intestinal fluid	<ul style="list-style-type: none"> <li>0.3% (w/v) Oxgall bile salts</li> <li>0.1% (w/v) pancreatin</li> <li>In saline solution (0.85% NaCl)</li> <li>pH 8.0</li> <li>Incubated at 37°C for 0, 30, 60, 120, 240, and 360 min</li> </ul>	

## RESULTS

Total CFU were concordant with the labeled number of cells for samples A, E, F and G. Sample C produced a lower CFU number per unit dose than that declared by the manufacturer. Total CFU originating from samples B, D, H, I and J were 1–3 log higher than those labeled. The amount of spores contained in samples A, C and F was concordant with the labeled amount of B. clausii spores and B. coagulans. (Table 2)

Formulation	Dose	Labeled cell no.	Total CFU	CFU from spores only	Formulation	Dose	Labeled cell no.	Total CFU	CFU from spores only
A	1 vial	2x10 <sup>9</sup>	1.15 ± 0.50x10 <sup>9</sup>	1.65 ± 0.71x10 <sup>9</sup>	F	1 bottle	1.25x10 <sup>11</sup>	2.16 ± 0.36x10 <sup>11</sup>	3.51 ± 1.49x10 <sup>10</sup>
B	1 capsule	2.4x10 <sup>10</sup>	2.71 ± 0.30x10 <sup>12</sup>		G	5 drops	5x10 <sup>9</sup>	9.65 ± 1.95x10 <sup>9</sup>	
C	1 bottle	2x10 <sup>9</sup>	6.02 ± 5.73x10 <sup>7</sup>	1.35 ± 0.50x10 <sup>7</sup>	H	1 capsule	3x10 <sup>9</sup>	5.74 ± 0.99x10 <sup>10</sup>	
D	5 drops	1x10 <sup>9</sup>	8.72 ± 1.53x10 <sup>11</sup>		I	1 sachet	2.97x10 <sup>11</sup>	3.51 ± 3.13x10 <sup>12</sup>	
E	1 capsule	5x10 <sup>9</sup>	2.68 ± 2.40x10 <sup>9</sup>		J	1 sachet	4.5x10 <sup>11</sup>	4.53 ± 0.47x10 <sup>12</sup>	

### Microbial survival in simulated gastric juice c

The majority of products showed a significant reduction ( $P < 0.05$ ) in the number of viable cells already after 30 min of incubation in the artificial gastric juice. The bacteria contained in samples A, I and J were found to be able to tolerate the acidic condition of the juice as long as 120 min. At this time, the survival of the organisms contained in these formulations was 96, 97, and 99%, respectively. (Figures 1 and 2).

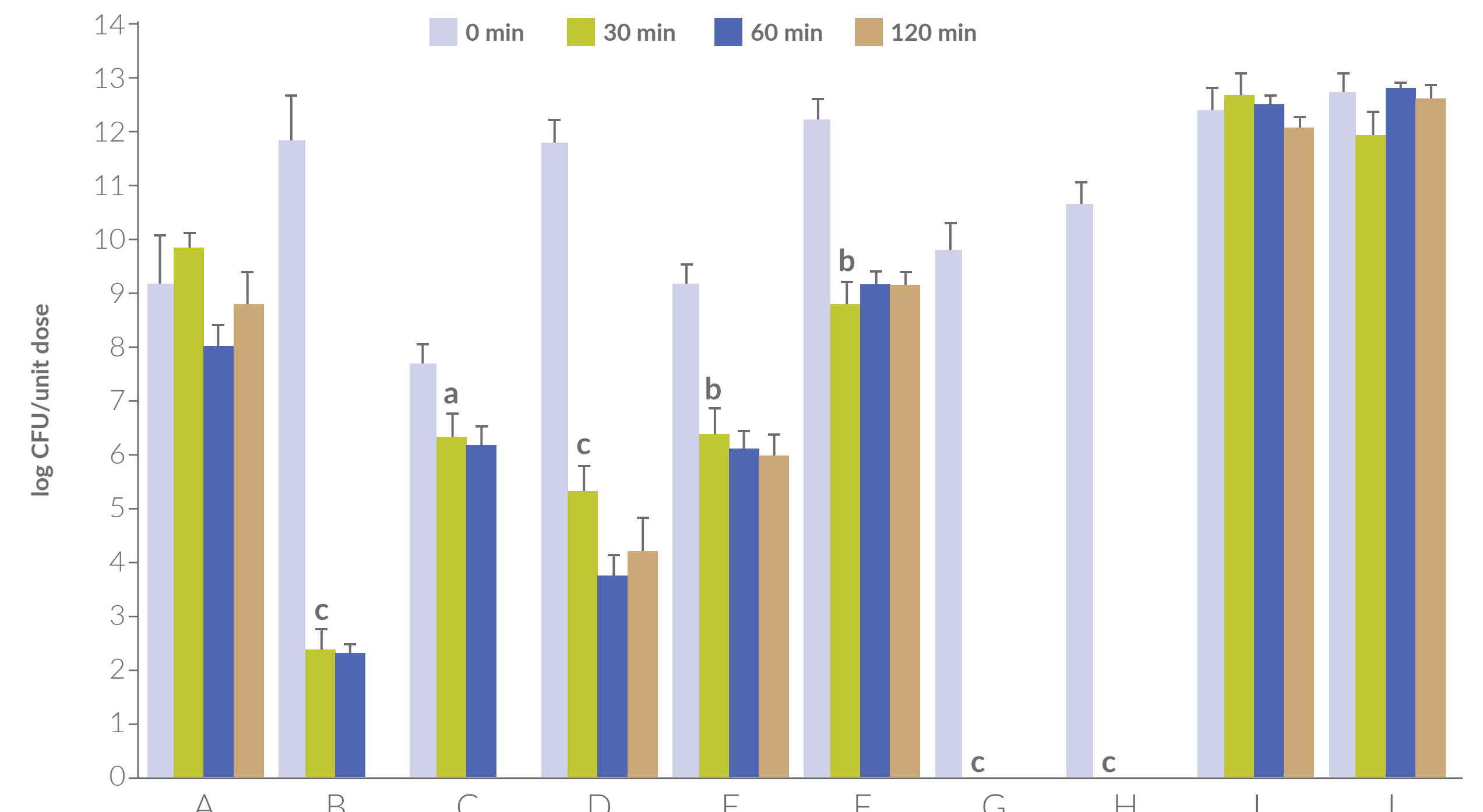


Figure 1. Viability of probiotic formulations in the ASTM-simulated gastric fluid. Microbial counts were carried out at 0, 30, 60, and 120 min and expressed as log CFU/unit dose of each product. aP < 0.05, bP < 0.01, and cP < 0.001.

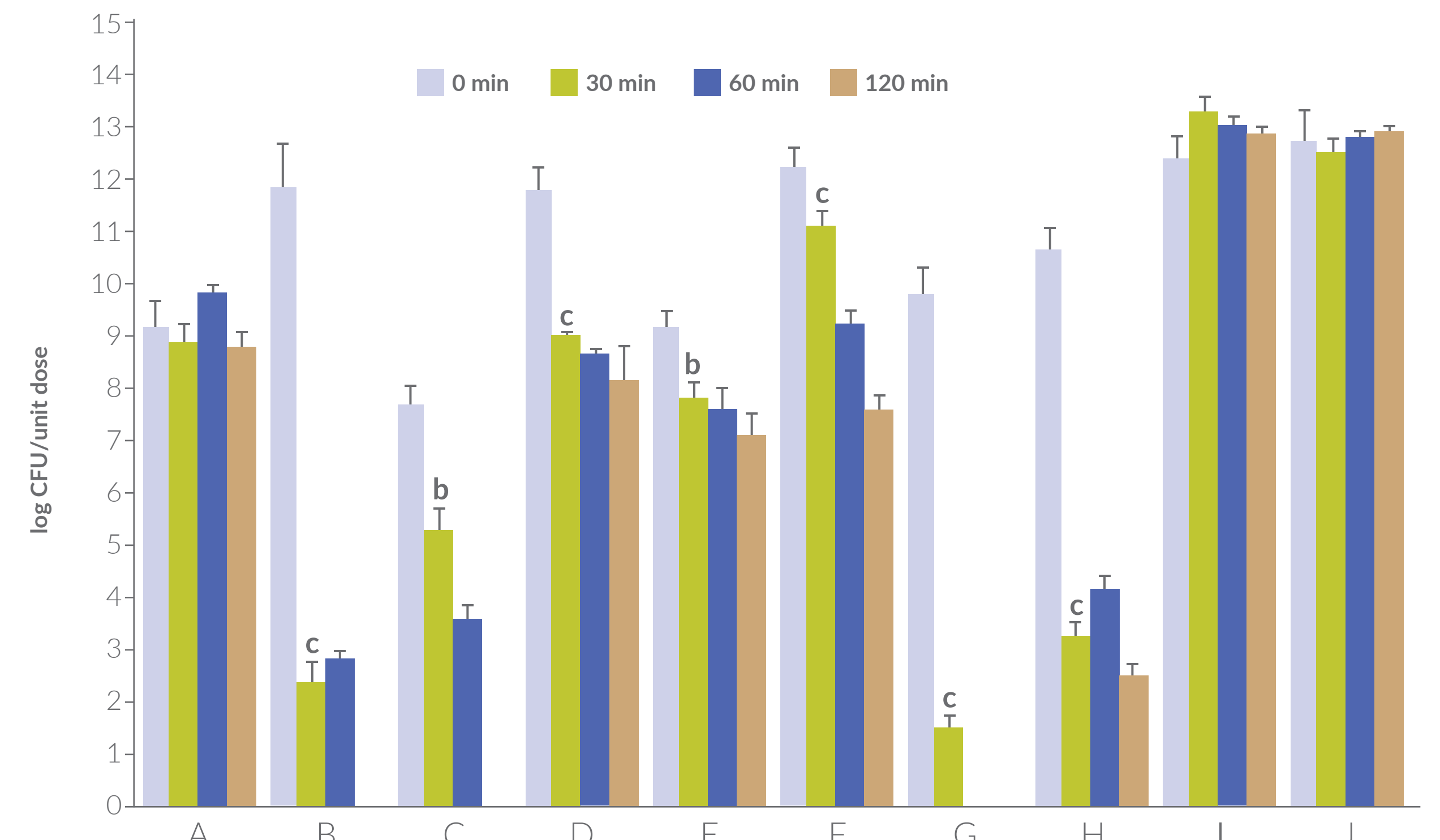


Figure 2. Viability of probiotic formulations in the U.S. Pharmacopeia simulated gastric fluid. Microbial counts were carried out at 0, 30, 60, and 120 min and expressed as log CFU/unit dose of each product. aP < 0.05, bP < 0.01, and cP < 0.001.

### Microbial Behavior in simulated intestinal juice c

A significant reduction in cell viability was recorded for samples B, D, F and G starting from 30 min of incubation, for samples I and J starting from 240 min, and for sample C at 360 min. No variation in cell viability was observed for sample H. Interestingly, the bacteria present in sample A were found able to replicate in the juice, with a significant increase in their number being recorded starting from 240 min of incubation. (Figure 3).

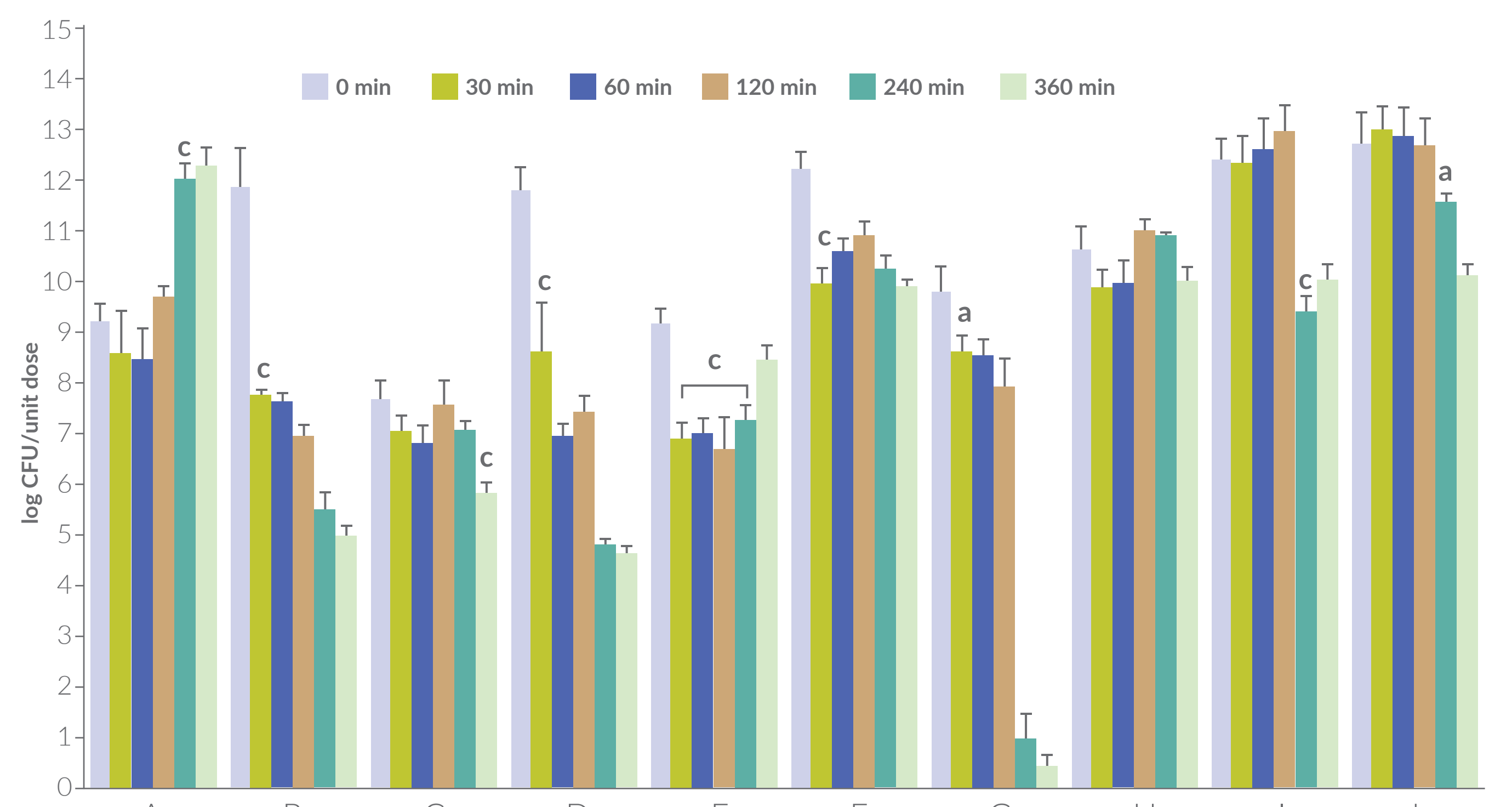


Figure 3. Behavior of probiotic formulations in simulated intestinal juice. Microbial counts were carried out at 0, 30, 60, 120, 240, and 360 min and expressed as log CFU/unit dose of each product. aP < 0.05, bP < 0.01, and cP < 0.001.

## CONCLUSIONS

- The results of this study indicate high quality of the examined probiotic preparations and highlight their different behavior in the presence of acid and bile.
- The amount of microorganisms contained in samples A, I and J is not reduced in these conditions for up to 2 h.
- The B. clausii spore suspension contained in sample A tolerate the acidic conditions of both ASTM and USP gastric juices well for 120 min.
- Moreover, B. clausii spores contained in sample A were found to be able to replicate in the intestinal juice, with a significant increase in the number of cells starting from 240 min of incubation. This finding correlates with previous data from an in vivo study on human volunteers indicating that sample A B. clausii (strains O/C, S/N, N/R, T) multiplies in the human intestine<sup>1</sup>.