Use of MicroSnap® Total and MicroSnap® EB Devices for Primary Incubation, Confirming by BAX® System PCR

Objective

This study aims to demonstrate the use of the MicroSnap® Total and MicroSnap® EB devices for incubation and indication of the presence of a select panel of bacteria (in this case, *Salmonella*). Then, confirm these organisms as viable and growing using BAX® System PCR methodology.

Materials & Methods

Materials

- MicroSnap Total
- MicroSnap EB
- BAX System Q7 Instrument
- BAX System Real-Time *Salmonella* (KIT2006)
- Pipettor and tips
- Incubators at 30 °C, 37 °C
- *Salmonella* Typhimurium ATCC 14028
- Tryptic Soy Broth (TSB)

Methods

Salmonella species were grown overnight in TSB at 37 °C. The *Salmonella* cultures were serially diluted 1:10 from the neat solution to a 10^{-9} dilution. For each test, 100 µl of each dilution was added in triplicate to either MicroSnap Total or MicroSnap EB enrichment swabs/devices. Devices were incubated at 30 °C and 37 °C, respectively.

The MicroSnap devices were analyzed at 6, 7, 8 and 16 hours. Triplicates were pooled and assessed via PCR at each of these time points.

Results

***Salmonella* Typhimurium Cultures**

For the *Salmonella* cultures, testing was performed using both methods. At 6 hours, results demonstrated that both the MicroSnap Devices and PCR could detect microorganism growth. However, the levels of detection varied between test methods (Table 1). MicroSnap Total detected positive growth at 1.2×10^{-2} dilution levels. However, PCR could detect growth at 1.2×10^{-3} dilution levels. Testing with the MicroSnap EB devices was more sensitive, with positive detection at a 1.2×10^{-3} dilution level, with PCR confirming a positive at the 1.2×10^{-4} dilution level.

Table 1: Growth Detection after 6 hours of Incubation

	CFU Added	MicroSnap Total Replicates 1,2,3 RLUs	Pooled PCR Positive/ Negative	MicroSnap EB Replicates 1,2,3 RLUs	Pooled PCR Positive/ Negative
Neat	1.2×10^{7}	555; 1,025;741	Positive	3,325;2,511;4,150	Positive
-1 dilution	1.2×10^{6}	1,022; 1,334; 455	Positive	4,410; 3,325;4,495	Positive
-2 dilution	1.2×10^{5}	123,44,56	Positive	1,277;995;635	Positive
-3 dilution	1.2×10^{4}	**5,11,3**	**Positive**	433, 152, 447	Positive
-4 dilution	1,220	1, 2, 1	Neg	**11, 9, 2**	**Positive**
-5 dilution	122	0, 1, 1	Neg	1, 2, 0	Neg
-6 dilution	19	0, 0, 1	Neg	1, 1, 1	Neg
-7 dilution	2	0, 0, 2	Neg	0, 1, 0	Neg
-8 dilution	0.2	0, 0, 0	Neg	0, 0, 0	Neg
-9 dilution	0.02	0, 0, 0	Neg	0, 1, 1	Neg

At 7 hours, similar results were obtained but with more sensitivity (Table 2). MicroSnap Total detected positive growth at the 1.2 x 10^{-4} dilution level. PCR was positive at the same dilution level. Testing with the MicroSnap EB devices was more sensitive, with positive detection at the 1.2 x 10^{-5} dilution level, with PCR confirming a positive down to the same dilution level.

Table 2: Growth Detection at 7 Hours of Incubation

	CFU Added	MicroSnap Total Replicates 1,2,3 RLUs	Pooled PCR Positive/ Negative	MicroSnap EB Replicates 1,2,3 RLUs	Pooled PCR Positive/ Negative
Neat	1.2 x 10^{7}	2,188; 2,411;1,944	Positive	6,114; 4,702; 5,361	Positive
-1 dilution	1.2 x 10^{6}	3,114; 2,551; 3,402	Positive	3,055; 4,632; 4,955	Positive
-2 dilution	1.2 x 10^{5}	563; 810; 125	Positive	2,092; 2,364; 2,555	Positive
-3 dilution	1.2 x 10^{4}	62;112;82	Positive	1,344; 994; 832	Positive
-4 dilution	1,220	**99, 124, 48**	**Positive**	52, 209, 314	Positive
-5 dilution	122	0, 0, 0	Neg	**52, 43, 145**	**Positive**
-6 dilution	19	0, 0, 2	Neg	3, 0, 1	Neg
-7 dilution	2	0, 0, 2	Neg	0, 2, 0	Neg
-8 dilution	0.2	1, 1, 2	Neg	0, 0, 1	Neg
-9 dilution	0.02	0, 0, 0	Neg	0, 1, 3	Neg

At 8 hours, MicroSnap Total detected positive growth at the 1.2 x 10^{-6} dilution level, consistent with PCR confirmation (Table 3). MicroSnap EB detected positive growth at the 1.2 x 10^{-7} dilution level and growth was confirmed by PCR at the same dilution level.

Table 3: Growth Detection at 8 Hours of Incubation

	CFU Added	MS Total Reps 1,2,3 RLUs	Pooled PCR Positive/ Negative	MS EB Reps 1,2,3 RLUs	Pooled PCR Positive/ Negative
Neat	1.2 x 10^{7}	610; 425;838	Positive	2,144; 3,051; 4,362	Positive
-1 dilution	1.2 x 10^{6}	1,245;1,552;2,010	Positive	8,412; 5,444; 6,188	Positive
-2 dilution	1.2 x 10^{5}	1,365; 1,892; 2,436	Positive	6,662; 5,894; 8,774	Positive
-3 dilution	1.2 x 10^{4}	1,459; 2,174; 1,566	Positive	3,251; 3,941; 2,584	Positive
-4 dilution	1,220	100; 259; 361	Positive	1,114; 522; 1,333	Positive
-5 dilution	122	321; 345; 112	Positive	1,451; 544; 1,247	Positive
-6 dilution	19	**91; 52; 109**	**Positive**	322; 1,422; 213	Positive
-7 dilution	2	1, 0, 0	Neg	**22, 59, 41**	**Positive**
-8 dilution	0.2	0, 0, 0	Neg	0, 0, 2	Neg
-9 dilution	0.02	0, 0, 0	Neg	0, 0, 1	Neg

At 16 hours, positive growth was confirmed by both MicroSnap devices at 10^{-7} dilution, with a few positive samples at 10^{-8} (Table 4). PCR confirmed growth at the 10^{-8} dilution level.

Table 4: Growth Detection at 16 Hours of Incubation

	CFU Added	MicroSnap Total Replicates 1,2,3 RLUs	Pooled PCR Positive/ Negative	MicroSnap EB Replicates 1,2,3 RLUs	Pooled PCR Positive/ Negative
Neat	1.2×10^7	522; 414; 395	Positive	1,841; 554; 622	Positive
-1 dilution	1.2×10^6	1,426; 2,555; 1,984	Positive	1,484; 2,594; 1,777	Positive
-2 dilution	1.2×10^5	1,423; 2,141; 2,201	Positive	5,448; 6,019; 4,788	Positive
-3 dilution	1.2×10^4	5,521; 4,871; 6,222	Positive	5,624; 6,471; 3,024	Positive
-4 dilution	1,220	1,412; 1,198; 852	Positive	1,554; 8,141; 2,503	Positive
-5 dilution	122	721; 1,215; 533	Positive	4,021; 5,023; 4,262	Positive
-6 dilution	19	1,123; 366; 548	Positive	5,144; 2,591; 5,002	Positive
-7 dilution	2	554; 2,195; 2,605	Positive	414; 203; 58	Positive
-8 dilution	0.2	**631, 0, 2**	**Positive***	**0, 0, 524**	**Positive**
-9 dilution	0.02	0, 1, 1	Negative	1, 2, 1	Negative

Discussion

This study was performed to determine if detectable microorganism growth in the MicroSnap Total or EB devices (in enrichment broth) could directly be transferred to a BAX System PCR assay to confirm *Salmonella* growth. Results indicate that the two systems are compatible. The enrichment media in the MicroSnap devices does not interfere with the detection of growth using the BAX System. Therefore, the two methods can be paired to confirm indicator organism growth and define the specific microorganism present, in this case, *Salmonella* Typhimurium.

Furthermore, the correlation between the intensity of the RLU signal detected with MicroSnap over time and microorganism growth was confirmed with the BAX System. With longer MicroSnap incubation times, the sensitivity of growth detection increased, and lower CFU levels can be verified using the BAX System.