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Application Note

Anaerobic Environmental Monitoring using MilliporeSigma lockable TSA + LTHTh ICR + plates

Ordering number: 1.46683.0020 / 1.46683.0120

Anaerobic environmental monitoring is not a routine process, but occasionally performed to detect anaerobic microorganisms related to contamination by personnel.

As *Propionibacterium* acnes is a common human skin commensal, it can act as a personal hygiene indicator. Furthermore it may be protected from decontamination, i.e. VHP treatment, because it is known as a biofilm associated microorganism. This strain is known to be a slow growing, anaerobic microorganism, which usually requires anaerobic incubation conditions and prolonged incubation times.

The aim of this study is to evaluate the effectiveness of incubating MilliporeSigma lockable TSA + LTHTh ICR+ plates at a higher than recommended incubation temperature, in order to increase the growth rate of *Propionibacterium* acnes and other anaerobic microorganisms, to support in-house validation of anaerobic monitoring:

1. Selection of plate design: Only lockable ICR+ plates, locked in the "VENT"-position provide sufficient gas exchange with the atmosphere of the anaerobic chamber to ensure reproducible growth conditions. The non-lockable plates or lockable plates in "CLOSED"-position may perform inconsistently.

2. Selection of the culture medium: In this study, the growth of selected anaerobic microorganisms was investigated on casein soya bean digest agar supplemented with the neutralizers lecithin, Tween® 80 (Croda Americas LLC, Wilmington, Delaware, US), histidine and sodium thiosulfate (TSA + LTHTh ICR+ plates). This medium is commonly used for aerobic environmental monitoring and was evaluated for its potential as a single medium to support both aerobic and anaerobic monitoring.

3. Selection of incubation temperature: Following the different guidelines, such as USP <1116>, incubation temperatures between 20 and 35 °C are recommended for environmental monitoring. In this study the growth of the selected microorganisms at an increased incubation temperature of 37 °C has been investigated in relation to the required incubation time.

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Methods and Material

Preparation of Test Strains and Inoculation

A single colony of the microorganism under test was transferred into brain heart infusion broth and cultivated at 34 °C for 16 to 18 hours. The freshly grown cultures of the test strains were diluted in NaCl Peptone Buffer to adjust the inoculum to values between 10 and 100 CFU on the reference plates. The inoculum was spread onto the surface of the reference plates (3 plates) and the test plates (2 plates) using BioPlater™5000 (BioSys GmbH).

Culture Media

Product name	Article number	Batch #
TSA + LTHTh ICR+	146683	130176
Columbia Blood Agar (100% reference)	146559	130343

Microbial Strains

Strain	Growth Pattern
Clostridium sporogenes ATCC 19404	anaerobic
Clostridium sporogenes ATCC 11437	anaerobic
Propionibacterium acnes ATCC 11827	anaerobic
Bacteroides fragilis ATCC 25285	anaerobic
Corynebacterium macginleyii in-house isolate	facultative anaerobic/aerobic

Incubation conditions

Temperatures: 30 °C or 37 °C
Duration for read out: 5, 7, 10 days
Atmosphere: Anaerobic (80%N_2, 10% CO_2, 10% H_2)

Results for Incubation Temperature 30 °C

Table 1: Recovery rates and colony sizes of microorganisms at anaerobic incubation at 30 °C on TSA + LTHTh ICR+ (VENT)

Strain	CFU Reference	CFU	Recovery (%)	Diameter of Colonies (mm)		
				after 5 days	after 7 days	after 10 days
C. sporogenes ATCC 19404	73/96/90 ⌀ 86	76/97	101	7 – 10	N/A	N/A
C. sporogenes ATCC 11437	48/49/59 ⌀ 52	56/52	104	5.5 – 10	N/A	N/A
P. acnes ATCC 11827	64/83/87 ⌀ 78	74/64	89	0.4	1.0 – 1.1	1.5
B. fragilis ATCC 25285	51/59/68 ⌀ 59	75/52	108	0.4 – 0.5	1.4 – 1.5	1.9 – 2.0
C. macginleyii (in-house isolate)	54/59/56 ⌀ 56	44/51	85	1.9	2.2 – 2.3	2.8 – 3.0

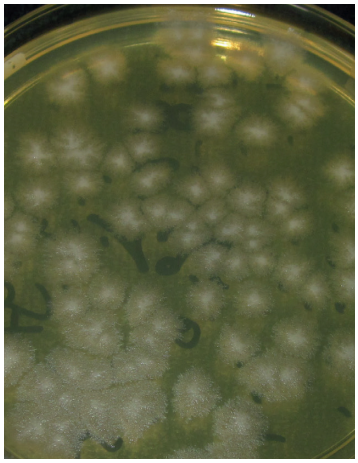

Fig. 1: C. *sporogenes* ATCC 19404

Fig. 2: C. *sporogenes* ATCC 11437

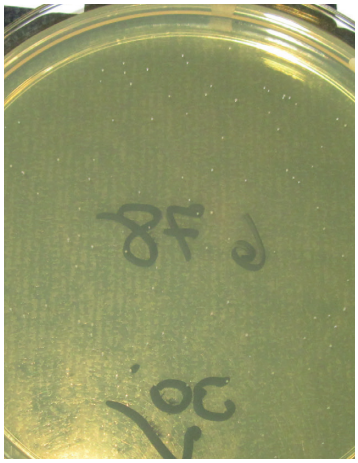

Fig. 3: *P. acnes* ATCC 11827

Fig. 1 – 5:
TSA + LTHTh ICR+ - Incubation 5 days incubation at 30 °C

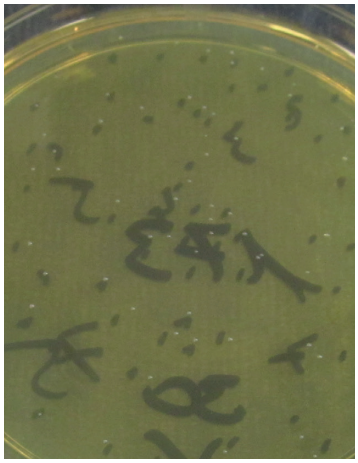

Fig. 4: *B. fragilis* ATCC 5285

Fig. 5: *C. macginleyii*

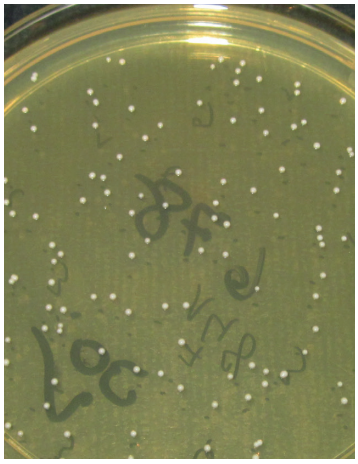

Fig. 6: *P. acnes* ATCC 11827

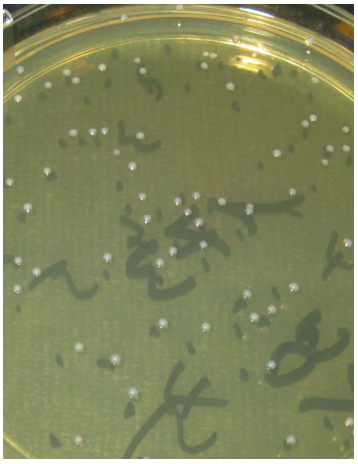

Fig. 7: ATCC 25285

Fig. 6 – 7:
TSA + LTHTh ICR+ - Incubation 7 days incubation at 30 °C

Results for Incubation Temperature 37 °C

Table 2: Recovery rates and colony sizes of microorganisms at anaerobic incubation at 37 °C on TSA + LTHTh ICR + (VENT

Strain	CFU Reference	CFU	Recovery (%)	Diameter of Colonies (mm)		
				after 5 days	after 7 days	after 10 days
C. sporogenes ATCC 19404	(111)/78/98 ø = 88	99/97	112	6 – 11	N/A	N/A
C. sporogenes ATCC 11437	42 / 56 / 47 ø = 48	58/47	110	5.5 – 12	N/A	N/A
P. acnes ATCC 11827	137 / 119 / 140 ø = 132	169/ 155	116	1.1	1.8	1.8
B. fragilis ATCC 25285	54 / 70 / 64 ø = 63	72/64	118	2.0 – 2.1	2.6 – 2.7	3.0 – 3.2
C. macginleyii (isolate)	ø = 45	42/51	103	1.8	2.5 – 2.8	2.8 – 2.9

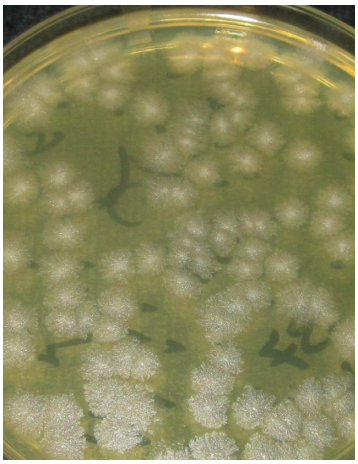

Fig. 8: *C. sporogenes* ATCC 19404

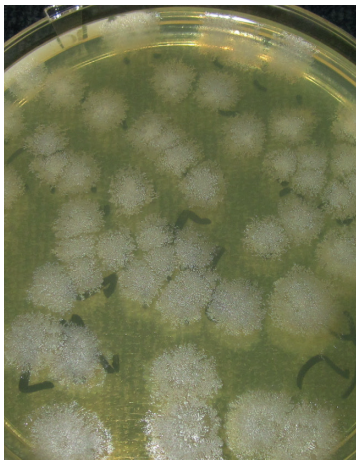

Fig. 9: *C. sporogenes* ATCC 11437

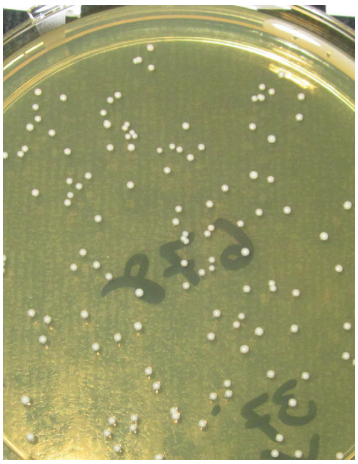

Fig. 10: *P. acnes* ATCC 11827

Fig. 8 – 12:
TSA + LTHTh ICR+ - Incubation 5 days incubation at 37 °C

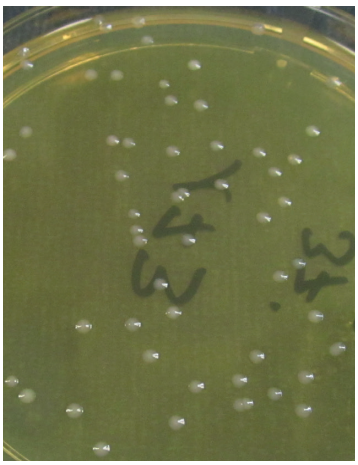

Fig. 11: *B. fragilis* ATCC 25285

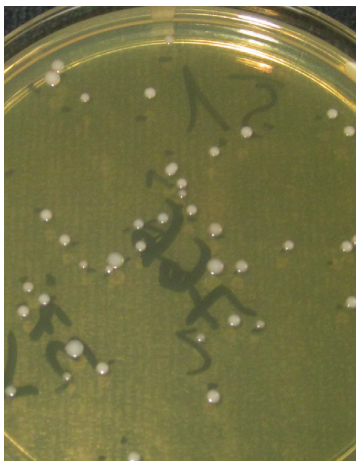

Fig. 12: *C. macginleyii*

Conclusion

The growth of all selected microorganisms under anaerobic conditions produced recovery rates of >50% at each incubation temperature. Increasing the temperature from 30 °C to 37 °C successfully increases the growth rate for *Bacteroides fragilis* and *Propionibacterium acnes*. At 30 °C, 7 days were required to achieve suitable colony sizes of *P. acnes* and *B. fragilis*, while 5 days at 37 °C already produced easily detectable colonies. Increasing incubation temperature to 37 °C did not significantly increase growth rates of Clostridia and *Corynebacterium macginleyii* as both organisms were easily detectable at both 30 °C and 37 °C incubation temperatures, following only 5 days incubation.

Compared to the more commonly used Chocolate Agar +LTH - ICR+ (article 146686) this study demonstrates that TSA + LTHTh is preferable for detection of the selected anaerobic microorganisms. *P. acnes* in particular, is known to grow slower on Chocolate Agar with LTH at 30 °C (> 7 days for achieving good visible colonies, data not shown) and 37 °C, producing smaller colonies compared to TSA + LTHTh.

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MilliporeSigma
290 Concord Road
Billerica, MA 01821
MilliporeSigma.com

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