

Introduction:

Enriched broth is recommended for the cultivation of facultative and obligatory anaerobic microorganisms present in clinical specimens. Copan developed the Thioglycollate enrichment Broth (THIOL), a ready to use LBM™ device, available in 4 ml in a PET tube with a cap with a plastic sealant to maintain anaerobic conditions during incubation and can be used with both manual and WASP™ automation streaking methods. The oxygen present inside the tube is eliminated by ingredients present in the formulation and supports the anaerobic condition during the incubation of clinical specimens.

The THIOL contains Vitamin K and Hemin as a growth supplements for many fastidious anaerobic bacteria.

Objective:

The objective of this study was to evaluate the Copan THIOL Broth for the enrichment of anaerobes using clinical anaerobes isolates.

Methods:

In this validation 32 anaerobic clinical strains of bacteria were used.

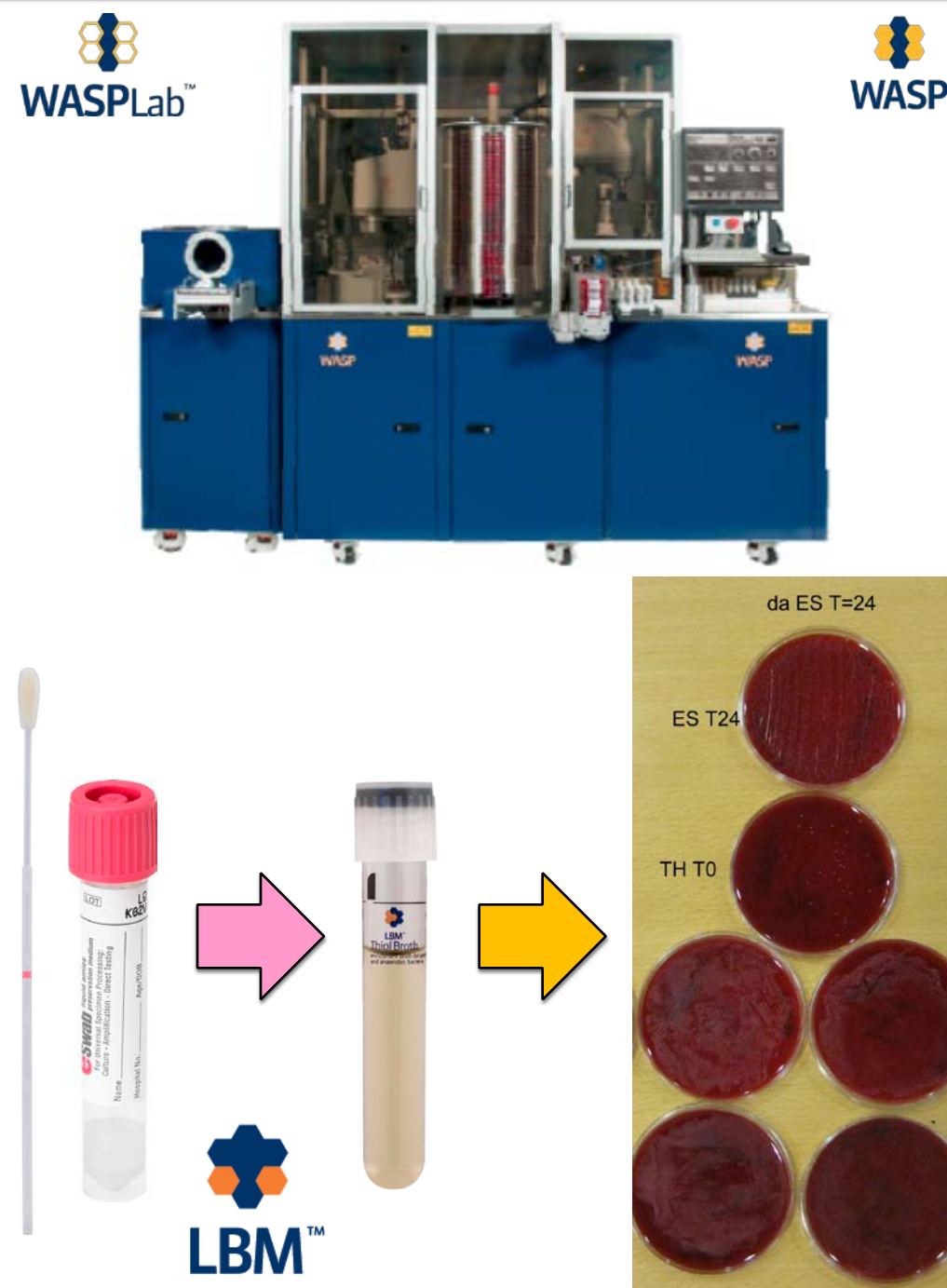
From a fresh culture for each clinical strain, a 0.5McFarland inoculum was prepared and further diluted to have a countable dilution containing 25-250ufc/100ul of Eswab™.

Aliquots of 100ul of each strain dilution were inoculated in ESwab™ kits, vortexed and 30ul of ESwab™ medium were inoculated in 2 different tubes of THIOL Broth by WASP™ automation at time zero and after 24 and 48 hours.

A 100ul aliquot of each strain at T0 was plated on Blood Agar with 5% sheep blood; plates were incubated at 35° C in anaerobic conditions.

All THIOL inoculated tubes were incubated at 35° C for 24 and 48 hours. After 24 and 48 hours all inoculated THIOL Broth were read for turbidity and a 100 ul aliquots of each THIOL Broth were plated as above.

Agar plates were incubated at 35° C in anaerobic conditions.



Results:

In the results obtained from the 32 clinical strains of anaerobes, 6 of the 32 strains were slow growing anaerobic bacteria, while 26 of the 32 strains were normal growing anaerobic bacteria.

Turbidity was present after 24 and 48 hours incubations in all inoculated THIOL Broth tubes confirmed by bacteria growth in the agar plates after 72 and 96 hours incubation for the slow growing and after 24 and 48 hours incubation for the normal growing bacteria.

Conclusions:

The results obtained demonstrated that the Copan THIOL supports slow and normal growing strains of anaerobic bacteria confirmed by the turbidity.

The PET material used for the tube and cap with a special sealant and the 4 ml medium volume are allowing optimal conditions for anaerobic bacteria growth.

Copan ESwab™ and THIOL broth can be used for the collection and enrichment of clinical specimens for the detection of slow and normal growing anaerobic bacteria with both manual and WASP™ and WASPLab™ automation broth inoculation and culture streaking.

Clinical Strains	Recovery from Thiol inoculated from Eswab at zero time			Recovery from Thiol inoculated from Eswab at time 24 h			Recovery from Thiol inoculated from Eswab at time 48h			Clinical Strains	Recovery from Thiol inoculated from Eswab at zero time			Recovery from Thiol inoculated from Eswab at time 24 h			Recovery from Thiol inoculated from Eswab at time 48h		
	T0	T24h	T48h	T0	T24h	T48h	T0	T24h	T48h		T0	T24h	T48h	T0	T24h	T48h	T0	T24h	T48h
<i>Anaerococcus murduchii</i>	2	++	+++	1	+	++	1	+	++	<i>Fusobacterium nucleatum</i>	3	66	122	1	99	189	1	99	222
<i>Anaerococcus octavius</i>	4	++	+++	1	+	++	1	++	++++	<i>Fusobacterium species</i>	2	103	208	1	112	217	0	88	305
<i>Atopobium parvulum</i>	3	+	++	1	+	+++	1	+	+++	<i>Fusobacterium naviforme</i>	3	97	311	2	106	257	0	95	238
<i>Bacteroides fragilis</i>	6	+++	++++	4	++	++++	4	++	++++	<i>Parabacteroides distasonis</i>	4	+	+++	4	++	+++	2	+	+++
<i>Bacteroides ovatus</i>	4	+++	++++	3	++	++++	3	++	++++	<i>Parvimonas micra</i>	2	79	228	1	81	303	0	109	231
<i>Bacteroides thetaiotamicron</i>	3	+++	++++	1	++	++++	1	++	++++	<i>Peptostreptococcus magna</i>	6	+++	++++	5	++	++++	5	++	++++
<i>Bacteroides uniformis</i>	3	+++	++++	1	++	++++	0	++	++++	<i>Peptostreptococcus species</i>	5	+++	++++	5	+++	++++	5	+++	++++
<i>Bacteroides vulgatus</i>	4	+++	++++	4	++	++++	2	+++	++++	<i>Porphyromonas somaree</i>	2	59	98	2	48	77	0	39	76
<i>Bilophila wadsworthia</i>	3	227	412	1	101	223	1	98	333	<i>Prevotella bivia</i>	3	126	402	1	157	501	0	203	489
<i>Blautia coccooides</i>	3	122	++	2	187	+	1	152	+	<i>Prevotella buccae</i>	2	135	208	1	119	302	0	98	239
<i>Clostridium perfringens</i>	4	++	++++	3	++	+++	3	+++	++++	<i>Prevotella disiens</i>	4	107	356	2	111	405	1	117	236
<i>Clostridium species</i>	6	+++	++++	3	++	++++	3	++	++++	<i>Prevotella nigrescens</i>	4	69	328	3	88	207	1	116	304
<i>Fingoldia magna</i>	7	+++	++++	5	+++	++++	4	+++	++++	<i>Prevotella oralis</i>	3	97	231	2	133	299	1	93	213
<i>Fusobacterium necroforum</i>	2	119	201	1	75	238	1	71	285	<i>Propionibacterium acnes</i>	5	+++	++++	4	+++	++++	4	+++	++++

Legend : + (2000.200 ufc /plate) ++ (4000 -2000 ufc /plate) +++ (semiconfluent colonies) ++++(confluent growth)