# Treponema

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## (九)

Normal Tris-HCl— EDTA fluid) Treponema (Reduced transport media saline Supplumented pleupneumolike organism New oral spirochetes) Trypton- yeast extract- Pepton- yeast extract- glucose Thioglycolate medium (glucose- volatile fatty acid-serum medium Trypton- yeast extract- glucose- volatile fatty acid-serum Thioglycolate-BHI agar) (agar Treponema (L-cystein Sodium thioglycolate) Fetal calf Rabitt serum Isobutyric acid Fetal bovin serum serum . Rifampcin Treponema .

# Detection of the Efficacy of some Transport, Isolation and Culture Media for Oral Treponema Isolated from Periodontitis

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## ABSTRACT

The ability of three types of transport media (Tris-HCl— EDTA fluid , Normal saline and Reduced transport media) to transfer oral treponema samples was investigated , in addition to the capability of relying five types of nutritional media (New oral spirochetes, Supplumented pleupneumolike organism, Thioglycolate medium, Pepton- yeast extract-glucose, and Trypton- yeast extract- glucose- volatile fatty acid-serum medium) and two types of solidified culture media (Thioglycolate-BHI agar and Trypton- yeast extract-glucose- volatile fatty acid-serum agar) for the primary isolation and subculturing of these organisms reaching the most necessary supplements required to support the growth of these organisms. The efficacy of the three types of transport media in transport and maintenance of the vaibilty of these organisms was shown, also the efficacy of primary isolation and subculturing media to support the organisms' growth was proved after supplementing these media with the necessary growth elements. Hence it was possible to provide the optimal anaerobic conditions for the growth by the addition of reducing agents (Sodium thioglycolate and L-cystein) to the culture media and incubation in anaerobic jar, furthermore, introducing the necessary elements of long chain fatty acid by the addition of isobutyric acid and serum, and we confirm the ability of three types of serum (Rabbit serum, Fetal calf serum and Fetal bovin serum) to support the culture media. We had showing possibility of depending upon the selection isolation method by the addition the antibiotic rifampicin. Moreover, it was observed two forms of growth of these organisms: the turbidity after two days of inocubation and the sediment form with the shot silk after four days of incubation, and the variation in colonies form on the solid media was also clear.

**Keywords**: Cultivation of oral Treponema, Nutritional requirements of oral Treponema .

Treponema Aggressive periodontitis Severe gingivitis Ulcerative gingivitis

Chronic periodontitis .(Kecis *et al*., 2008 ; Edwards *et al*., 2005) Periodontal tissues Treponema Treponema Thiamin pyrophosphate (TTP) Cystenyl-glysin (Cys-Gly) Cysteine Serine Histidin ( Tucci and Martin, 2007 ; Fenno *et al*., 2000) .Methionine Glutathione Well – reduced anaerobic media Treponema Gas tank Gas pak 0.1% .(Prescott *et al*., 2005 ; McDowell *et al*., 2005)

Treponema Wells method - : Treponema Treponema Membrane filters method 0.22) Treponema Treponema 0.7% Treponema .(Starr *et al*.,1981) Patience Treponema

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**Samples**

Periodontitis (Kasuga *et al.*, 2000)

- Cotton roll
- Cotton pellet Supragingival plaque
- Periodontal probe Pocket depth (PD)
- Paper point 0.5

**Transport media**

**: Tris-HCl— EDTA (T-E) Fluid .1**

DTA (Ethylen (Desvarieux *et al.*, 2005) Tris-HCl 10 diamine tetra acetic acid)

**: Normal saline 2**

(Willis *et al.* ,1999)

**:Reduced transport media 3**

0.5 Brain heart infusion 37 (Umeda *et al.*, 1990)

Distilled water Sodium thioglycolate

15 121 7.2

**Culture media**

: Treponema

**1. New oral spirochetes (NOS) (Uitto *et al.,* 1995):**

Brain heart infusion 12.5: Sodium thioglycolate 0.5 Yeast extract 2.5 Trypticase 10.0 Glucose 0.006 L-Aspartate 2.0 L-Cysteine 0.25 Ascorbic acid 1.0 Sodium bicarbonate 2.0 Distilled water.

**2. Supplumented pleupneumolike organism (PPLO) (Difco manual,1998):**

Beef extract 50.0 : Sodium 2.0 Sodium thioglycolate 0.5 NaCl 5.0 Pepton 10.0 Distilled water Crystal violet 0.01 Bicarbonate 1.0 :(Starr *et al.,*1981) Treponema Ascorbic acid 0.006 Nicotinamide 0.4 L-Cysteine 1.0 Glucose.

**3. Thioglycolate medium:**

30 MERAEK D+ 5.5 Yeast extract 5.0 Pepton 15.0 : Glucose 0.001 Sodium thioglycolate 0.5 NaCl 2.5 L+ Cysteine 0.5 Agar-agar 0.75 Resazurin sodium.

**4. Pepton- yeast extract- glucose (PYG):**

Pepton 20.0 :(Lennett *et al.,*1985) Sodium 0.5 Glucose 10.0 L-Cysteine 0.5 Yeast extract 10.0 $^3$ 960 Salts solution $^3$ 40.0 Sodium bicarbonat 2.0 Thioglycolate Distelled water.

$MgSO_4$ 0.2 $CaCl_2$ 0.2 : Salts solution 300 $MgSO_4$ $CaCl_2$ $KH_2PO$ 1.0 $K_2HPO_4$ 1.0 $^3$ 200 500 .

**Trypton- yeast extract- glucose- volatile fatty acid- serum (TYGVS) [illegible] 5**

**medium** :[illegible]

5.0 に Trypton [illegible] 20.0 : [illegible] (Starr *et al.,*1981) [illegible] 0.5 に L-Cysteine [illegible] 0.75 に NaCL [illegible] 2.5 に Glucose [illegible] 5.0 に Yeast extract [illegible]

. Distilled water [illegible] に Sodium bicarbonate [illegible] 2.0 に Sodium thioglycolate [illegible] (Wyss, 1992) TTP [illegible] Ascorbic acid [illegible] PPLO μANOS [illegible] Sodium thioglycolate [illegible] 0.5 [illegible] PPLO, PYG, TYGVS [illegible] μAg [illegible] μA / [illegible] 2.0 [illegible] Sodium bicarbonate [illegible] μA [illegible] μAg [illegible] . (Hardy and Munro,1966) [illegible]

**: Treponema** [illegible]

[illegible] : (Starr *et al.,*1981)

**: Thioglycolate-BHI agar [illegible] 1**

Brian (1 Part) に (3 Parts) Thioglycolate medium : [illegible]

(%0.7) Agar-agar に Heart infusion

**: TYGVS agar [illegible] 2**

. %0.7 [illegible] μg [illegible] TYGVS broth [illegible]

Isobutyric acid [illegible] 7.2 [illegible] %0.2 [illegible] 100 / [illegible] 0.2 [illegible] Rifampcin [illegible] (Uitto *et al.,* 1995) %2

.(Leshine and Canale-Parola, 1980) [illegible]

:[illegible] Treponema [illegible]

**: Rabbit serum [illegible] .1**

[illegible] 10 [illegible] (Izard *et al*., 2004) [illegible] μg [illegible] μA [illegible] . [illegible]

**: Fetal calf serum (FCS) [illegible] .2**

20 (Wyss *et al.,* 2004) Jugular vein

**:Fetal bovin serum (FBS) .3**

10 3000 0.2 30 56 (Starr *et al.,* 1981)

**:**

0.1 Thioglycolate NOS, PPLO, PYG, TYGVS Oxiod Anaerobic jar Anaerobic indicator (Resazurin) Vaccume $(N_2)$ $(CO_2)$ %80 %10 37 1

$N_2$

$CO_2$

1

**:Treponema** [illegible]

[illegible]

[illegible]

**:** [illegible]

[illegible] Spirochetes [illegible] (Collee *et al.*,1996) [illegible] 1000X [illegible]

[illegible]

**:** [illegible]

Reduced BHI broth [illegible] Normal saline [illegible] T-E [illegible] *In vitro* [illegible] Treponema [illegible] (2) [illegible] 5- 4 [illegible] (3) [illegible] (4) [illegible]

NOS [illegible]

PYG [illegible]

TYGVS [illegible]

[illegible]

[illegible] Treponema [illegible] 2 [illegible]

(-) : [illegible] (+) : [illegible]

[illegible] Treponema [illegible] 3 [illegible]

NOS [illegible] :C [illegible] PYG [illegible] :B [illegible] TYGVS [illegible] :A

[illegible] 4 [illegible] NOS [illegible]

[illegible] Treponema [illegible] (Socransky *et al.*, 1969) [illegible]

[illegible] (Wolf *et al.*, 1993) [illegible] Treponema [illegible]

Club-like [illegible] Plaits [illegible] structure [illegible] B- 2 [illegible] Cell Pellets [illegible] (Wyss *et al*., 1996) [illegible] End granules [illegible] ABHI [illegible] NOS [illegible] PPLO [illegible] Yeast extract [illegible] Trypticase [illegible] PYG [illegible] Thioglycolate [illegible] Beef extract [illegible] TYGVS [illegible] Isobutyric acid [illegible] Sodium thioglycolate [illegible] $H_2S$ [illegible] L- cysteine [illegible] Treponema [illegible] Anaerobic jar [illegible] $N_2$ [illegible] $CO_2$ [illegible] Resazurine [illegible] (5) [illegible]

[illegible] 5 [illegible]

PPLO (1964), (1969) Socransky Treponema (Umemoto *et al.,*1988) Ascitic fluid 4 Treponema PPLO 37

Thioglycolate broth Treponema TYGVS Umemoto *et al.,* (1997) TYGVS Thioglycolate medium TYGVS (Lux *et al.*, 2002) (Miyamoto *et al.*, 2006) Treponema

Treponema NOS (Limberger *et al.*, 1999) (Edwards *et al.*, 2003)

Treponema PYG (1976) , Johnson *In vitro* Treponema

Base medium

Treponema Ascitic fluid Treponema

[illegible] Human serum [illegible] (Hardy and Munro, 1966) [illegible] Fetal calf serum (FCS) [illegible] Fetal bovin serum (FBS) [illegible] Horse serum [illegible] (Wyss,1992 ; Izard *et al*., 2004) [illegible] Sodium thioglycolyate [illegible] Isobutyric acid [illegible] Socransky [illegible] Treponema [illegible] Treponema [illegible] 1964 [illegible] Eh [illegible] Treponema [illegible] -190mv) [illegible] Fusiform [illegible] Putrescin [illegible] Diphtheria [illegible] Isobutyric acid 0.002) Sodium thioglycolate [illegible] Treponema [illegible] .(0.25) Putrescin dihydrochloride [illegible] Dithiothreitol [illegible] Treponema [illegible] Brooks *et al*., 2007; Yoshid *et al*., ) [illegible] Eh [illegible] .(2004

[illegible] Treponema [illegible] (1966) Munro [illegible] Hardy [illegible] Isobutyric Acid [illegible] Isobutyic acid [illegible] 10 [illegible] Treponema [illegible] (Wyss,1992) [illegible] .

[illegible] NOS [illegible] 2 [illegible] Sodium bicarbonate [illegible] Treponema [illegible] TYGVS [illegible] YG [illegible] PLO [illegible] Bicarbonate [illegible] Treponema [illegible] Diphtheriods [illegible] Lag Phase [illegible] Isobutyric acid [illegible] Sodium bicarbonate [illegible] $CO_2$ [illegible] .(Hardy and Munro, 1966 ; Chan *et al.*,1993) [illegible]

[illegible] L-cysteine [illegible] Protease [illegible] Cysteine [illegible] Treponema [illegible] . (Chu *et al.,* 2002)

[illegible] Treponema [illegible] (Lai and Chu, 2008) [illegible] $H_2S$ [illegible] Glutathione [illegible] L-Cysteine [illegible] $H_2S$ [illegible] %0.6 - 0 [illegible] Sulfate [illegible] $H_2O$ [illegible] Cysteine [illegible] $H_2S$ [illegible]

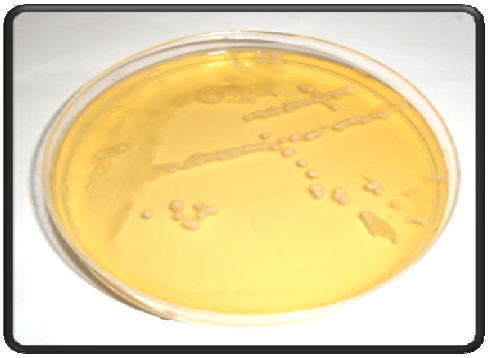
[illegible] (Wyss, 1992) [illegible] TPP [illegible] Ascorbic acid [illegible] Treponema [illegible] 1 [illegible] Ascorbic acid [illegible] .

[illegible] Treponema [illegible] 00/ [illegible] 0.2 [illegible] Rifampcin [illegible] Treponema [illegible]

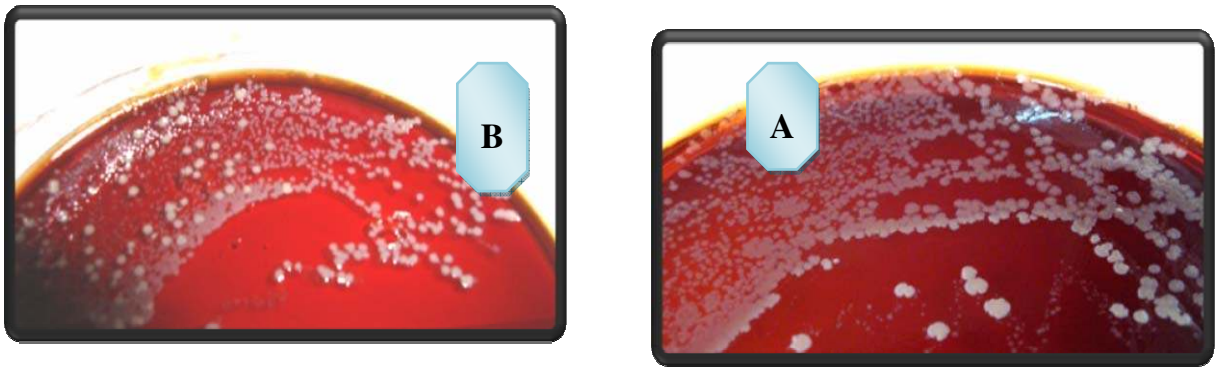
[illegible] Leschine and ) [illegible] .(Canale-parola, 1980

DNA – dependent [illegible] β – Subunit [illegible] Rifampcin [illegible] *poβ* gene [illegible] RNA Polymerase (Rpoβ) [illegible] Rpoβ [illegible] Treponema [illegible] .(Stamm *et al.,* 2001) [illegible]

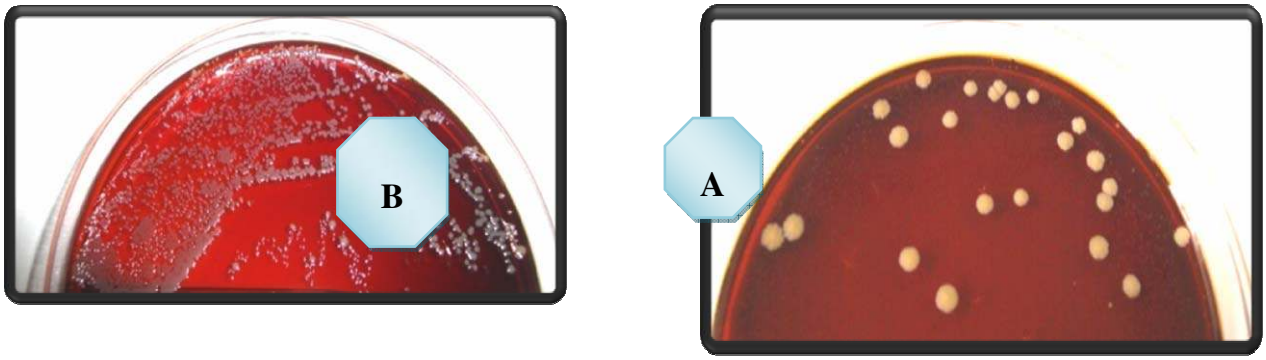
**Treponema** [illegible] Thioglycolate-BHI agar [illegible] TYGVS agar [illegible] %0.7 [illegible] 3≤ [illegible] (6) [illegible] TYGVS agar [illegible] (7) [illegible] Thioglycolate-BHI agar [illegible] (8) [illegible] (9) [illegible] .Treponema [illegible]



.TYGVS Agar [illegible] Treponema [illegible] .6 [illegible]




Thioglycolate-BHI Agar Treponema 7 A B

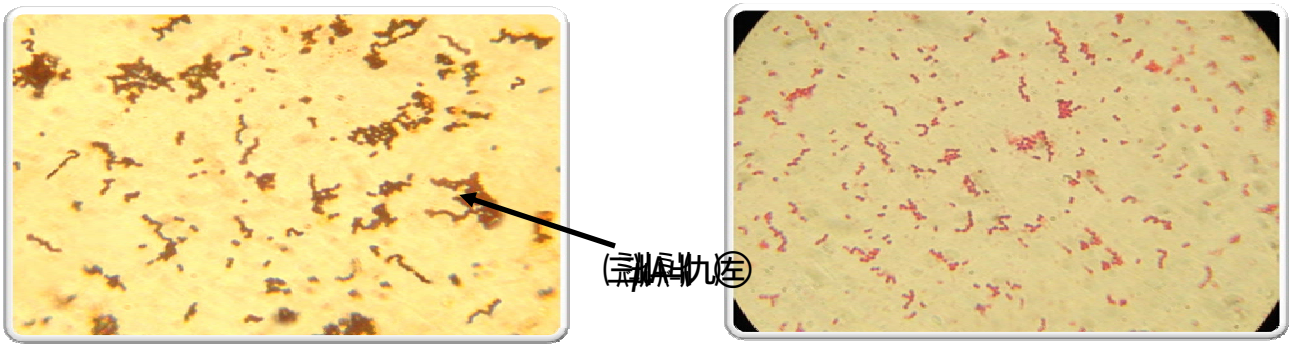



Thioglycolate-BHI Agar Treponema 8 B A

Thioglycolate –BHI Treponema 9 Agar

Treponema [illegible] PPLO [illegible] (Socransky *et al.*, 1964) [illegible] 1.2 [illegible] PPLO [illegible] Treponema [illegible] Subsurface colonies [illegible] Surface colonies [illegible] (Starr *et al.*, 1981) [illegible] 0.7 [illegible] PPLO [illegible], (1982) Nauman [illegible] Jacob [illegible] 10% [illegible] (1980) Canale-Parola [illegible] Leschine [illegible] 3 [illegible] Treponema [illegible] (Umemoto *et al.*, 1984) [illegible] Transparent [illegible] 5 [illegible] 15 –5 [illegible] (Wardle, 1997) [illegible] kg [illegible] (10) [illegible]

Breeding nests (11).



Treponema 10 1000X.

Treponema 11 1000X.

Irregular Lossely wounds Tightly coiled coild Borrelia Treponema Leptospira Levaditi Fontana (Collee *et al*., 1996; Goering *et al*., 2008)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