

Treponema

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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 vaibility 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

Treponema anaerobic media

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

Samples

Periodontitis

(Kasuga *et al.*, 2000)

/

:

- . Cotton roll •
- . Cotton pellet Supragingival plaque •
- . Periodontal probe Pocket depth (PD) •
- . Paper point •
- . ³ 0.5

Transport media

:

: Tris-HCl— EDTA (T-E) Fluid .1

DTA (Ethylen 1 (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 1 Sodium thioglycolate

15 °121

7.2

Culture media

:

: *Treponema*

: **New oral spirochetes (NOS)** .1

Brain heart 12.5: (Uitto *et al.*, 1995)

Sodium thioglycolate 0.5 Yeast extract 2.5 Trypticase 10.0 infusion
 0.006 Glucose 2.0 L-Aspartate 0.25 L-Cysteine 1.0
 . Distilled water 1 Sodium bicarbonate 2.0 Ascorbic acid

: **Supplumented pleupneumolike organism (PPLO)** .2

Beef extrac 50.0 : (Difco manual,1998)

Sodium 2.0 Sodium thioglycolate 0.5 NaC l 5.0 Pepton 10.0
 .Distilled water 1 Crystal violet 0.01 bicarbonate
 1.0 :(Starr *et al.*,1981) *Treponema*

. Ascorbic acid 0.006 Nicotinamide 0.4 L-Cysteine 1.0 Glucose

: **Thioglycolate medium** .3

30 MERA EK

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

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

Pepton 20.0 :(Lennett *et al.*,1985)

Sodium 0.5 Glucose 10.0 L-Cysteine 0.5 Yeast extract 0.0
³ 960 Salts solution³ 40.0 Sodium bicarbonat 2.0 thioglycolate
 . Distilled water

MgSO₄ 0.2 CaCl₂ 0.2 : Salts solution

³ 300 MgSO₄ CaCl₂ KH₂PO 1.0 K₂HPO₄ 1.0
³ 200 ³ 500

Trypton- yeast extract- glucose- volatile fatty acid- serum (TYGVS) . 5

: **medium**

5.0 Trypton 30.0 : (Starr *et al.*,1981)

0.5 L-Cysteine 0.75 NaCL 2.5 Glucose 5.0 Yeast extract

. Distilled water 1 Sodium bicarbonate 2.0 Sodium thioglycolate

(Wyss, 1992) TTP Ascorbic acid PPLO NOS

Sodium thioglycolate 0.5 PPLO, PYG, TYGVS

/ 2.0 Sodium bicarbonate

. (Hardy and Munro,1966)

: **Treponema**

: (Starr *et al.*,1981)

: **Thioglycolate-BHI agar .1**

Brian (1 Part) (3 Parts) Thioglycolate medium :

(%0.7) Agar-agar heart infusion

: **TYGVS agar .2**

. %0.7

TYGVS broth

Isobutyric acid

7.2 %0.2

³ 100 / 0.2 Rifampicin (Uitto *et al.*, 1995) %2

.(Leshine and Canale-Parola, 1980)

: Treponema

:

: **Rabbit serum .1**

³ 10 (Izard *et al.*, 2004)

: **Fetal calf serum (FCS) .2**

.....

³ 20 (Wyss *et al.*, 2004)

Jugular vein

:Fetal bovin serum (FBS) .3

10 / 3000

0.2 30 ° 56

.(Starr *et al.*, 1981)

:

³ 0.1

Thioglycolate NOS, PPLO, PYG, TYGVS

Oxid

Anaerobic jar

Anaerobic indicator (Resazurin)

Vaccume

(N₂)

%80 (CO₂)

%10

5

°37

(1)

N₂



:1

:Treponema

:

Spirochetes

(Collee *et al.*,1996)

1000X

:

Reduced BHI broth Normal saline T-E

In vitro

Treponema

(2)

5-4

(3)

(4)



NOS



PYG



TYGVS

.....

Treponema :2

(-) :

(+) :



Treponema :3

NOS :C PYG :B TYGVS :A



.NOS

:4

Treponema

(Socransky *et al.*, 1969)

(Wolf *et al.*, 1993)

Treponema

Club-like

Plaits

structure

3-2

Cell Pellets

(Wyss *et al.*, 1996)

End granules

BHI

NOS

PPLO

Yeast extract

Trypticase

PYG Thioglycolate

Beef extract

TYGVS

Isobutyric acid

Sodium thioglycolate

H₂S

L- cysteine

Treponema

Anaerobic jar

N₂ CO₂

.(5)

Resazurine



.....

PPLO (1964), (1969) Socransky
 Treponema
 (Umemoto *et al.*, 1988) Ascitic fluid
 4 Treponema %5 PPLO
 . 37

Thioglycolate broth Treponema
 TYGVS Umemoto *et al.*, (1997) TYGVS
 Thioglycolate medium
 TYGVS (Lux *et al.*, 2002)
 (Miyamoto *et al.*, 2006) Treponema
 . 4

Treponema NOS
 %0.5 %10 (Limberger *et al.*, 1999)
 (Edwards *et al.*, 2003)
 . %15

Treponema PYG
 (1976) , Johnson
In vitro Treponema

15-14 Base medium
 .
) %2
 . (Treponema
 Ascitic fluid
 Treponema

Human serum (Hardy and Munro, 1966)

Fetal calf serum (FCS) Fetal bovin serum (FBS) Horse serum
 . (Wyss, 1992 ; Izard *et al.*, 2004)

Sodium thioglycolate

Isobutyric acid

Socransky Treponema
 Treponema 1964

Eh Treponema
 . (-190mv)

Fusiform

Putrescin Diphtheria Isobutyric acid
 0.002) Sodium thioglycolate Treponema
 .(³ / 0.25) Putrescin dihydrochloride (³ /

Dithiothreitol

Treponema

Brooks *et al.*, 2007; Yoshid *et al.*,) Eh
 .(2004

Treponema

(1966) Munro Hardy Isobutyric Acid

Isobutyric acid

%10

Treponema (Wyss, 1992)

.....

NOS

/ 2

Sodium bicarbonate

Treponema

TYGVS

PYG

PPLO

Bicarbonate

Treponema

Diphtheriods

Lag Phase

Isobutyric acid

Sodium bicarbonate

CO₂

.(Hardy and Munro, 1966 ; Chan *et al.*,1993)

L-cysteine

Protease

Cysteine

Treponema

. (Chu *et al.*, 2002)

Treponema

(Lai and Chu, 2008)

H₂S

Glutathione

L-Cysteine

H₂S

%0.6 - 0

Sulfate H₂O

Cysteine

H₂S

(Wyss,1992)

TPP

Ascorbic acid

Treponema

/

1

Ascorbic acid

Treponema

³

100/

0.2

Rifampcin

Treponema

Leschine and)

.(Canale-parola, 1980

DNA – dependent

β – Subunit
rpo β gene

Rifampicin
RNA Polymerase (Rpo β)

Rpo β

Treponema

.(Stamm *et al.*, 2001)

Treponema

Thioglycolate-BHI agar

TYGVS agar

%0.7

3 \leq

-

(6)

TYGVS agar

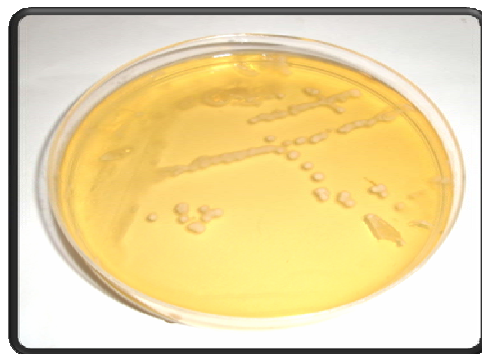
(7)

Thioglycolate-BHI agar

(8)

(9)

.Treponema

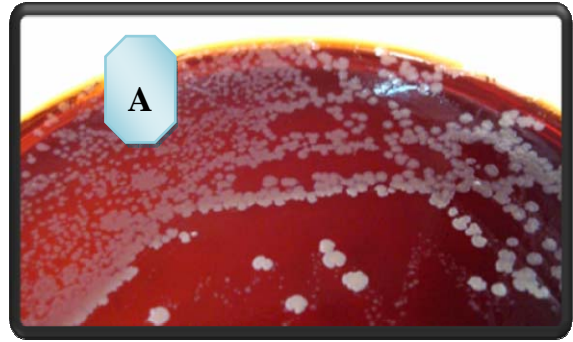


.TYGVS Agar

Treponema

:6

.....



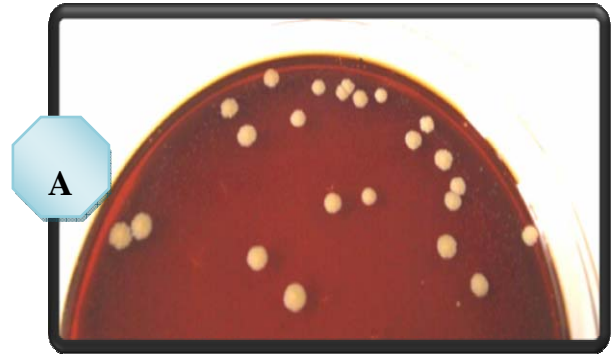
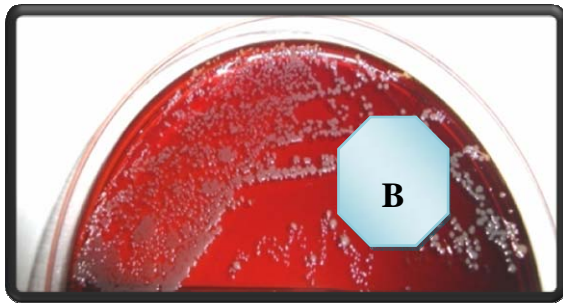
Thioglycolate-BHI Agar

Treponema

:7

- : A

- : B



Thioglycolate-BHI Agar

Treponema

:8

- :B : A



Thioglycolate -BHI

Treponema

:9

Agar

Treponema PPLO (Socransky *et al.*, 1964)

% 1.2 PPLO

Treponema

Subsurface colonies

Surface colonies

(Starr *et al.*, 1981)

% 0.7

PPLO , (1982) Nauman Jacob

%10

(1980) Canale-Parola Leschine

3

Treponema

(Umemoto *et al.*, 1984) .Transparent

5

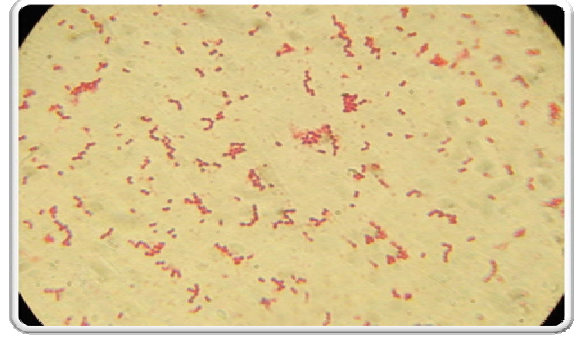
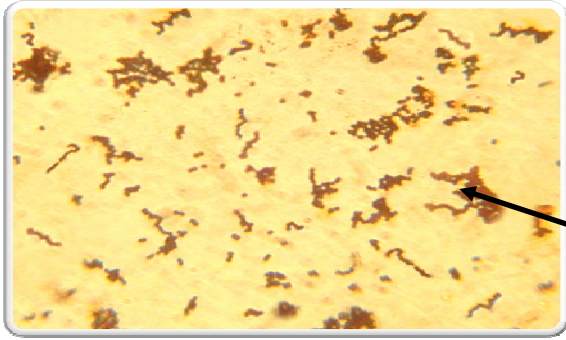
15 -5

.(Wardle, 1997)

(10)

Breeding nests

.(11)



Treponema :11
.1000X

Treponema :10
.1000X

Irregular

Lossely wounds

Tightly coiled

coild

Borrelia

Treponema

()

Leptospira

Levaditi

(

) Fontana

.(Collee *et al.*, 1996; Goering *et al.*, 2008)

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