جامعة الملك سعود
King Saud University

# LECTURE (2)


## Parasites Culture &
## Cultivation Of Luminal Parasitic Protists

# PARASITIC CULTURE

Parasite cultivation used to study the biochemistry, physiology, and metabolism of the parasite, determine their nutritional requirements, understand their ultra-structural organization, as well as assess functional antibodies and cell-mediated protective systems against the parasites, and also provides a system to assess vaccine efficacy.

# DIFFICULTIES IN CULTIVATION OF PARASITES

1. Most parasites have complex life-cycles with different morphological stages and may have both cold-blooded and warm-blooded animals as hosts within the life cycle. These stages involve number of variables including parasitic form, host site, host temperature, host immune responses, parasite species and/or strain, and parasite-protective mechanisms. To stimulate the host environment, especially in an *in vitro* culture system can be extremely demanding, assuming one can actually determine all the relevant variables.

2. Parasites are often fastidious and require medium components that may be toxic.

3. Filter sterilization may be required in some cases.

4. Human or animal sera, which are expensive and highly variable are usually required for successful culturing of parasites. In some cases, growth factors have been identified and substituted for serum.

# Three types of culture media

**Xenic culture**

It refers to culture of parasites grown in association with unknown microbiota.
Ex. Stool specimens cultured for *E. histolytica* in National Institute of Health medium. It is used for primary growth of parasites.

**Monoxenic culture**

It refers to culture of parasites with a single known bacterium.
Ex. Corneal biopsy specimens cultured with *Escherichia coli* as a means of recovering species of *Acanthamoeba*. It can be used for primary growth as well as a transitional phase in isolation.

**Axenic culture**

It is a pure culture without any bacterial associate or any other metabolizing cells. It is mainly used as isolation medium for the parasites.
Ex. TYI-S-33 medium in case of *T. vaginalis*.

# General principles:

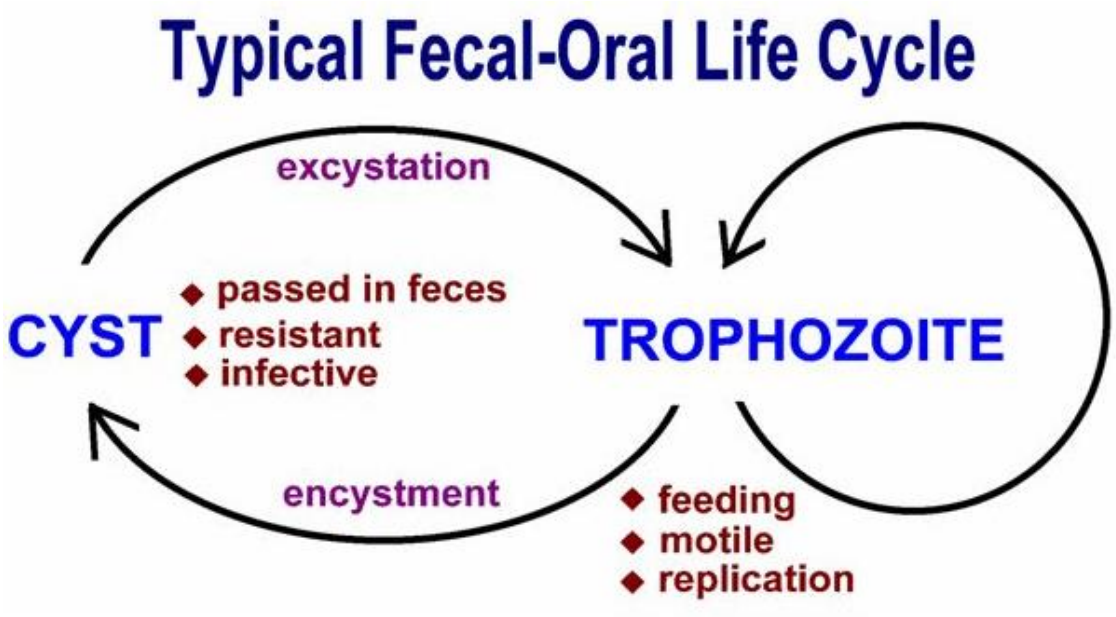
Although the province of parasitic cultivation is very diverse, there are certain principles which are applicable at large to the subject:

1. Parasitic helminths are **MORE DIFFICULT** to cultivate than protozoa. The complexity of helminth body configuration and metabolism, and inability to meet essential environmental conditions account for failure to complete their life-cycles under artificial conditions.
2. Cell cultures are used for **OBLIGATE INTRACELLULAR PARASITES**, for example *Plasmodium* spp. and coccidian.
3. Various kinds of **NUTRIENTS** as blood, serum, haem, egg, peptone, minerals and carbohydrates used in culture media.
4. Temperature required for **OPTIMUM GROWTH** is usually $37^0$C though lower temperatures may be required in few cases, e.g. 25-$27^0$C for *Leishmania* promastigotes.
5. Incubation condition is **AEROBIC** with some exceptions like microaerophilic conditions for *Amoebae* and *Giardia* and 5% CO2 for *Plasmodium* spp.
6. **IDENTIFICATION** tools include parasite's characteristic morphology, direct fluorescent antibody assay, polymerase chain reaction, enzyme immunoassay, etc.
7. Positive **CONTROLS** need to be run in parallel to keep a check on the medium and the method used.

# CULTIVATION OF LUMINAL PARASITIC PROTISTS

# Luminal Parasitic Protists

…. Protozoa colonize and infect the oro-pharynx, duodenum and colon.
…. They are transmitted by the fecal-oral route (food/water).




…. Outbreaks of diarrhea and dysentery are especially problematic in daycare centers.
…. The cyst forms of protozoa are resistant to chlorine and ozone and can become important when the municipal water supply is overburdened with these organisms

Flagellates:
- ***Giardia lamblia***
- *Dientamoeba fragilis*
- *Chilomastix mesnili*
- *Trichomonas hominis*
- *Enteromonas hominis*
- *Retortamonas intestinalis*

Ameba:
- ***Entamoeba histolytica***
- *Entamoeba dispar*
- *Entamoeba coli*
- *Entamoeba hartmanni*
- *Endolimax nana*
- *Iodamoeba bütschlii*

Apicomplexa:
- *Cryptosporidium parvum*
- *Cyclospora cayetanensis*
- *Isospora belli*

Microsporidia:
- *Enterocytozoon bieneusi*
- *Encephalitozoon intestinalis*

Other:
- *Blastocystis hominis*
- *Balantidium coli*

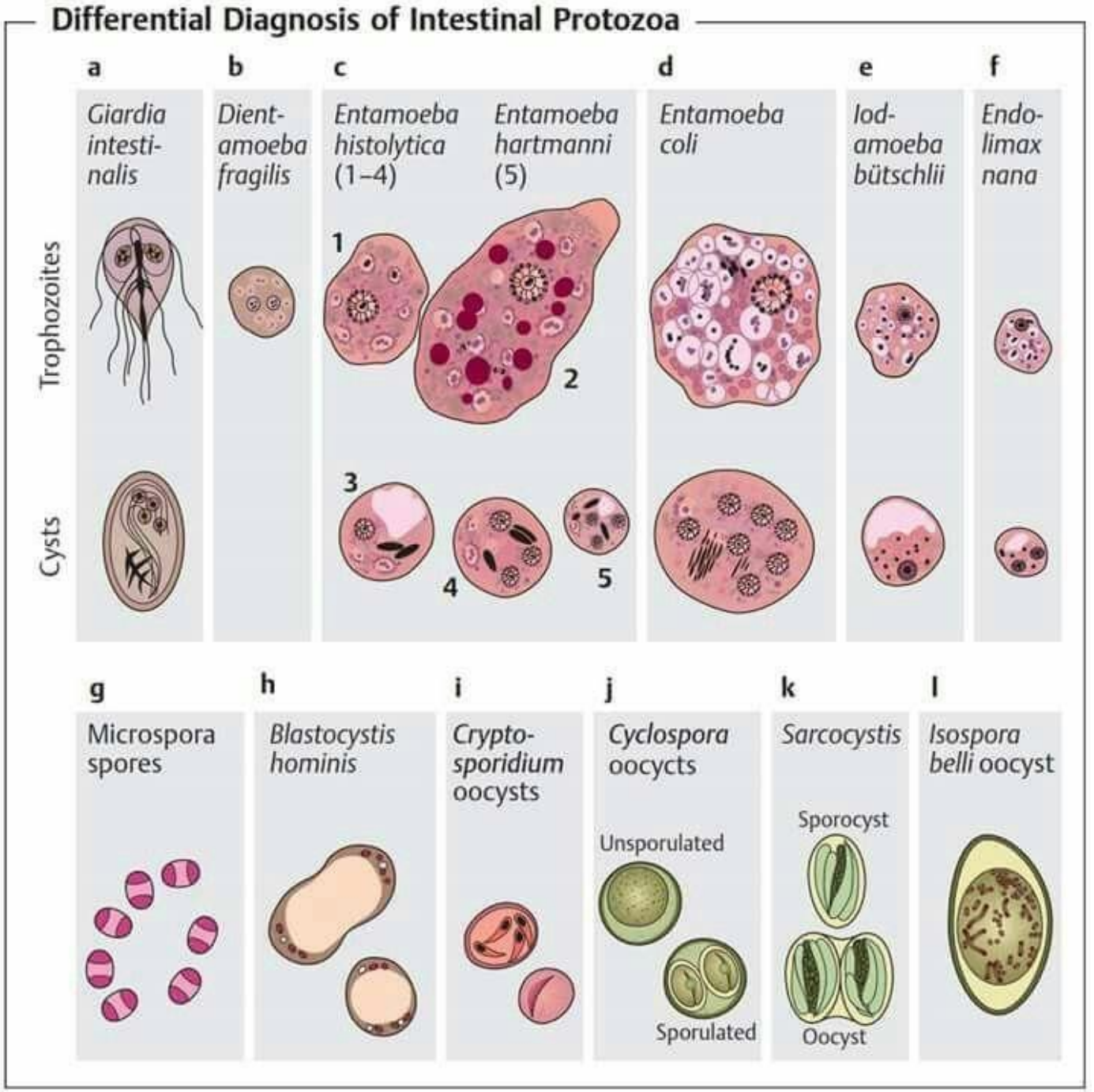
# INTESTINAL PROTOZOA

## • Pathogenic

- *Entamoeba histolytica*
- *Balantidium coli*
- *Giardia lamblia*
- *Dientamoeba fragilis*
- *Cryptosporidium parvum*
- *Enterocytozoon bieneusi*
- *Septata intestinalis*
- *Cyclospora cayetanensis*
- *Isospora belli*

## • Commensal

- *Entamoeba hartmani*
- *Entamoeba dispar*
- *Entamoeba coli*
- *Endolimax nana*
- *Iodamoeba bütschlii*
- *Chilomastix mesnili*
- *Trichomonas hominis*
- *Blastocystis hominis*



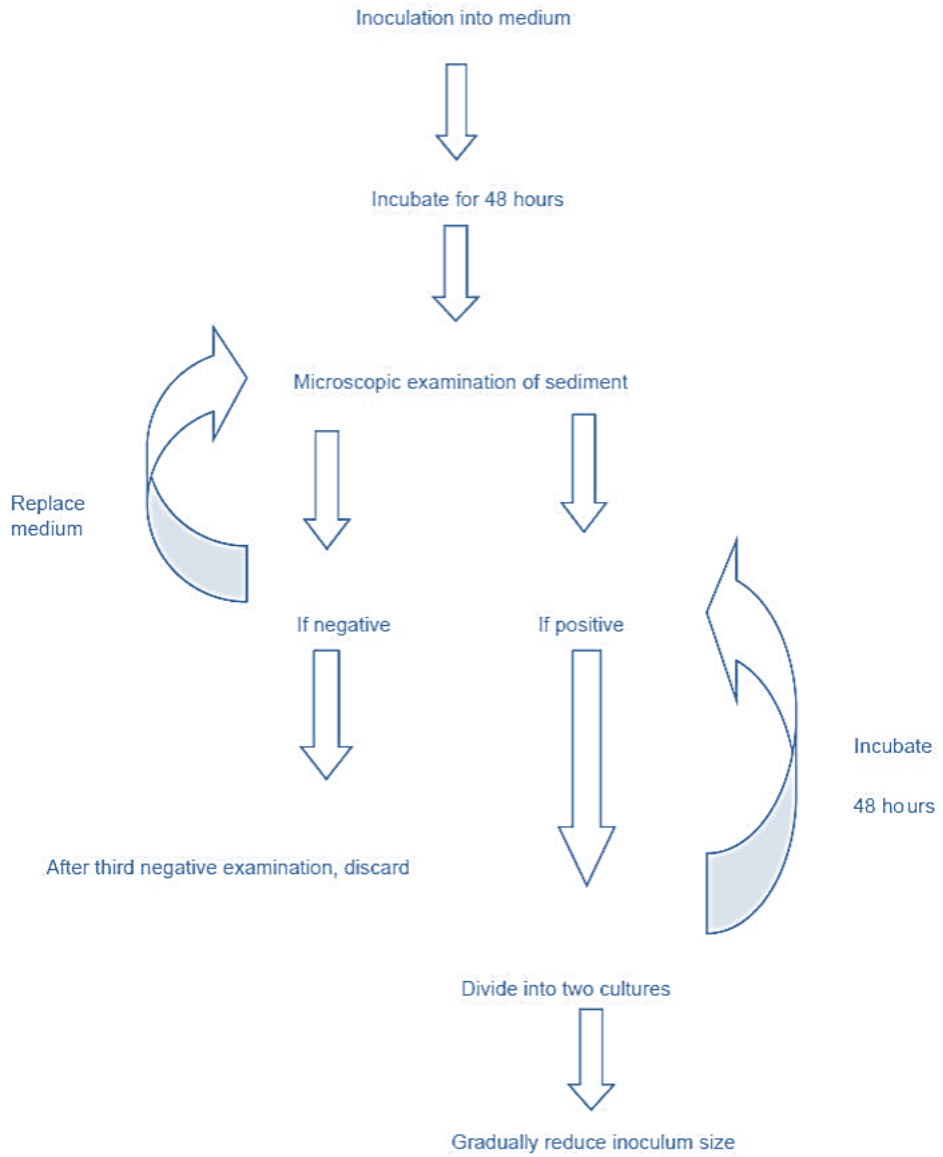
# Maintenance of cultures

…. Established cultures of all parasites are handled largely in the same way.

…. **XENIC CULTURES** of *E. histolytica*, *D. Fragilis*, *B. hominis* and *B. coli* are passaged at 48–72 h intervals. Xenic cultures should be passaged using two or more inoculum sizes to ensure a successful subculture.

…. **AXENIC CULTURES** of *T. vaginalis*, *E. histolytica*, *G. intestinalis*, and *B. hominis* are passaged at 72 and 96 h intervals. For doing subculture:

1. cultures are **chilled** in an ice-water bath to release trophozoites attached to the glass culture tube.
2. tubes need **not be chilled** unless an accurate count is desired. Tubes are inverted several times to disperse the cells and a measured inoculum is passed aseptically to a culture tube containing fresh medium.




Flow diagram illustrating the stages in establishing luminal parasites in culture

## Media for cultivation of luminal parasitic protists

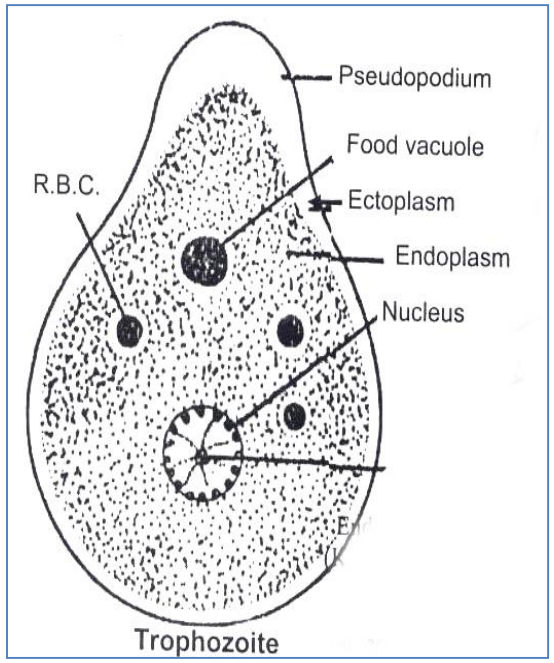
| Medium | Type | Components/ conditions | Parasites cultivated | Uses/ remarks |
|---|---|---|---|---|
| Balamuth's | Xenic liquid | Phosphate buffer<br>Whole liver concentrate solution<br>Egg yolk | *Entamoeba histolytica* | Used for studying the effects of drugs, amebicides and antibiotics |
| Boeck and Drbohlav's | Xenic diphasic | Locke's solution<br>Eggs<br>Inactivated human serum | *Entamoeba histolytica, Balantidium coli* | NIH modification of Boeck and Drbohlav's Medium: LE medium |
| Cleveland's | Monoxenic diphasic | Thioglycollate, penicillin inhibited *Streptobacilli* | *Entamoeba histolytica, Entamoeba coli, Trichomonas vaginalis* | Shaffer and Frye's modification |
| Diamond's | Axenic liquid | Trypticase<br>Yeast extract<br>Inactivated bovine serum<br>Minerals | *Entamoeba histolytica, Giardia intestinalis, Trichomonas vaginalis* | TYI-S-33 modification; large yield of pure culture; modified for other protists |
| Linstead's | Axenic defined medium | Antibiotics and antifungals | *Trichomonas vaginalis* | "InPouch TV" for transport and culture |

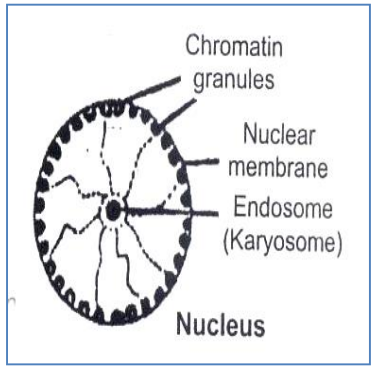
LE: Locke-egg

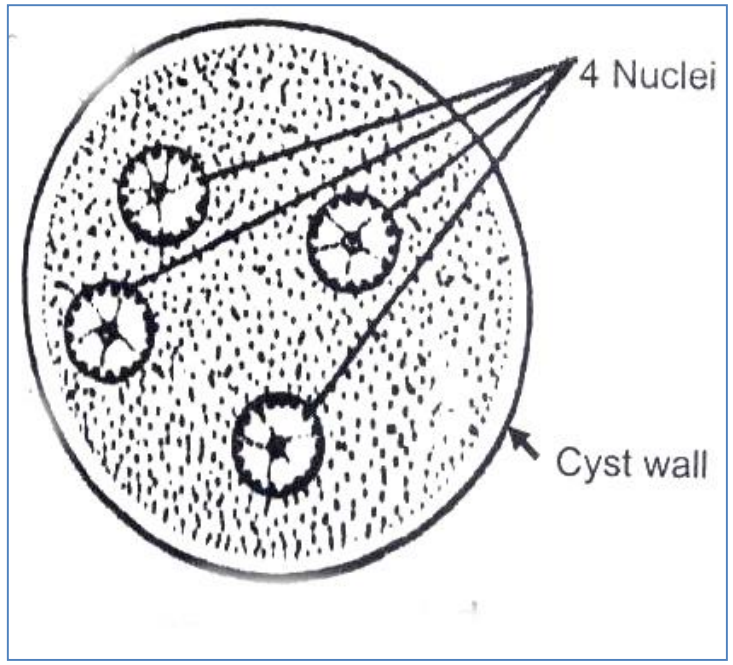
# ***Entamoeba histolytica* culture**

…. It is the agent of intestinal and hepatic amebiasis, can be cultivated in conjunction with the bacteria voided in feces by the infected patient.

…. It could be cultivated by both xenic and axenic methods.




Two stages of *E. histolytica* which are trophozoite and cysts

# I- Xenic cultivation for *Entamoeba histolytica*

## Boeck and Drbohlav's Locke –Egg Serum (LES) medium

…. The medium is basically an egg slant, with an overlay of sterile serum or liver extract in buffered saline.
…. Cultures can be obtained from feces containing cysts or trophozoites.

**Preparation of complete medium**

1. Wash 4 eggs, wipe the shells with 70% alcohol, and break eggs into a sterile flask containing glass beads.
2. Add 50 ml of **Locke's solution** and shake until homogenous.
3. Dispense medium so that a slant of 1:1 inches is produced in tube bottom .
4. Plug tubes and place them in a slant position in an inspissator at 70C until slant solidifies.
5. Tubes can then be autoclaved for 20 minutes. Discard any damaged slants.
6. Prepare a mixture of 8 parts sterile Locke's solution to 1 part inactivated human serum.
7. Sterilize the mixture by filtration and incubated at 37$^{O}$C for 24 to 48 hrs as a sterility check before use. Cover the slants to a depth of 1 cm with the sterile solution and inoculate. LES medium should have a loopful of sterile rice powder with fresh feces or its saline centrifugal sediment added before inoculation.
8. Antibiotics are also added to the medium to inhibit the over growth of various organisms. These include; penicillin (1000units/ml) streptomycins 2 mg/ml and acriflavine (0.1 ml of 0.02%).

**Locke solution (Auto-Clave before storage)**

Nacl: 9 gm
CaCl2: 0.2 gm
KCL: 0.4 gm
NaHCO3: 9.0 gm
Glucose: 2.5 gm
Distilled water: 1000 ml

The culture examine 0.1 ml of sediment under the microscope for characteristic motility.

Although the initial culture may appear to be negative, subcultures may reveal organisms.

# II- Axenic cultivation for *Entamoeba histolytica*

## TYI-S-33 medium

### TYI broth

1.0 g of potassium phosphate, dibasic
0.6 g of potassium phosphate, monobasic
2.0 g of sodium chloride
20.0 g of casein digest peptone
10.0 g of yeast extract
10.0 g of glucose
1.0 g of L-cysteine hydrochloride
0.2 g of ascorbic acid
1.0 ml of ferric ammonium citrate, brown form (22.8 mg/ml)

→ dissolve in 600 ml of deionized or glass-distilled water

Bring the final volume to 880 ml and pH to 6.8 using 1 N sodium hydroxide solution.
Dispense in 88-ml amounts into 125-ml glass bottles and autoclave for 15 min at 121°C
Sterile TYI base can be stored frozen at 20°C.

### Vitamin mix 18

Dissolve 45 mg of niacinamide, 4 mg of pyridoxal hydrochloride, 23 mg of calcium pantothenate, 5 mg of thiamine hydrochloride, and 1.2 mg of vitamin B12 in 25 ml of water.

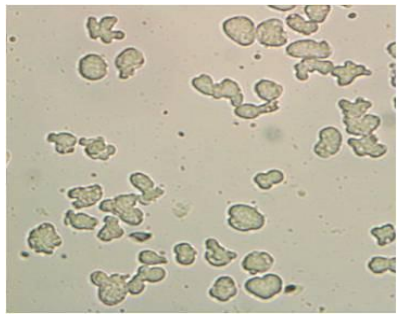
+ Dissolve 7 mg of riboflavin in water using the minimum amount of 0.1 N sodium hydroxide, bringing the final volume to 45 ml.

+ Dissolve 5.5 mg of folic acid in water using the minimum amount of 0.1 N sodium hydroxide, bringing the final volume to 45 ml.

+ Dissolve 2 mg of D-biotin in water bringing the final volume to 45 ml.

+ Dissolve 1 mg of DL-6-8-thioctic acid (oxidized form) in 5 ml of 95% ethanol. Add 500 mg of Tween 80, bringing the volume to 30 ml with water.

Combine solutions, bring final volume to 200 ml with dist. H2O, and sterilize through a 0.22-m-pore-size filter. Store in 100-ml amounts at 4°C for up to 6 months. The solution is light sensitive. The above recipe provides enough vitamin mix 18 to make 10 liters of complete TYI-S-33.



*E. histolytica* culture

Add 2.0 ml of vitamin mix 18 and 10 to 15 ml of heat-inactivated adult bovine serum to each 88 ml of TYI broth. Dispense into screwcap borosilicate glass culture tubes (16 by 125 mm). The tubes should be filled to ca. 80% capacity (including inoculum of *E. histolytica*).

thank you