Global Journal of Medical research
Interdisciplinary
Volume 13 Issue 7 Version 1.0 Year 2013
Type: Double Blind Peer Reviewed International Research Journal
Publisher: Global Journals Inc. (USA)
Online ISSN: 2249-4618 & Print ISSN: 0975-5888

Trichrome Stain for Diagnosis of Amoebae in Parasitology Laboratory

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Abstract - For many years the trichrome staining technique (TricrómicWheatley) has been considered as the most important technique for the identification ofthe most common intestinal protozoa and popularin parasitology (1).

Currently the mostsensiblidad procedure for detecting and identifying protozoa trophozoites stool sample as it helps to easily highlight the morphology of amoebic cysts and trop-hozoites however, the procedure is complicated and tediousto perform and require at seven different reagents which is probably the most critical especially in laboratories with limited staff, this makes it complicated the routine use of this technique in most of the clinical laboratory, using koplic additionally facilitates reagent contamination by repeated use.(4,5)

Keywords : *tricrómicwheatley, diagnosis of amoebae.*

GJMR-K Classification : *NLMC Code: WF 141*

Strictly as per the compliance and regulations of:

Trichrome Stain for Diagnosis of Amoebae in Parasitology Laboratory

Coloración Tricromica Para El Diagnostico De Amebas En El Laboratorio De Parasitologiaclinica

DaissyJ Vargas Sepúlveda

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Resumen- Por muchos años la técnica de coloración tricromía (TricrómicWheatley) ha sido considerada como la técnica más importante para la identificación de protozoos intestinales la más común y popular en parasitología (1).

Actualmente es el procedimiento con mayor sensiblidad para detectar e identificar trofozoitos de protozoarios en muestra de materia fecal ya que ayuda a evidenciar fácilmente la morfología de quistes y trofozoitos de amebas sin embargo, el procedimiento es complicado y tedioso de realizar y requiere siete reactivos diferentes lo cual es probablemente lo mas critico especialmente en laboratorios con personal limitado, esto hace que sea complicado el uso rutinario de esta técnica en la mayor parte de los laboratorio clínicos, adicionalmente el uso de koplic facilita la cont- aminación de los reactivos por el uso repetido. (4, 5)

Palabras clave: coloración tricromica, diagnostico de amebas.

I. Introduction

The main purpose of this study was to evaluatea new method for obtaining atrichromic staining faster and effective for it used the samedyesand proce-ededim plementing two different technical koplicone with and one with direct drops of reagent in the lamina.

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There were 20 positive smearsall parasites and made several technical modifications in order to simplify and expedite the procedure equally maintaining the excellent staining qualities, the nimplemented the steps mentioned in the original technique and then the technique modified.

Original Technical Steps

Wheatley's Modification of the Gomori Trichrome stain

1. Schaudinn 30 minutes
2. 70% Ethanol 5 minutes.
3. Place slide in70% ethanol iodado al 1 minute.
4. Place slide in 70% ethanol for 5 minutes
5. Place slidein 70% ethanol for 3 minutes in other Koplic.
6. Trichrome stain 10 minutes
7. Destain in 90% ethanol y acétic acid por 1 a 3 seconds
8. Rinse several times in 100% ethanol
9. Place in two changes of 100% ethanol for 3 minutes each
10. Place in two changes of xylene or xylene substitute for 10 minutes
11. Mount with coverslip using mounting medium (e.g., permount).
12. Examine the smear microscopically utilizing the 100× objective. Examine at least 200 to 300 oil immersion fields

Step in the technique modified

1. Perform aslidesheetth in extended Let dryat room temperature
2. Add saturated mercury chloride for 20 minutes
3. Add Trichrome stain + alcohol 96% for 10 minutes
4. Wash and add 96% ethanol 4 minutes
5. Examine the smear microscopically utilizing the 100×objective.

Recommendations for modified technique

1. The most important step for aisgood results is the fixation of the sample, because if not set the samplemay shrink orprotozoamaytakean abnormal color identification difficult.

2. The smear should not be too thick to facilitate identification of cysts and trophozoites.
3. You must completely remove mercury or Schaudin in the first step of coloring because if left too much, it tends to form crystalsor granules that can prevent the identification of any organism.
4. After adding trichrome bleaching should be performed in a short timeasmay appear washed staining is likely due to excessive discolouration.
5. May. And the final stage of dehydration with 100% ethanol should be as free of water as possible to avoid both the reactive evaporation of moisture absorption as that can preventeasy identification of the parasite.(2)

Note: formalin fixed Fecal samples are suitable for this dyeing process

a) Important considerations

The fund continues to see green and cytoplasm of protozoa is stained a blue green and purple. There dnuclei with inclusions purple and intracellular structures are easy to distinguish as glycogen vacuoles are the Iodamoeba butschlii. (6)

Experimental development

Validation of the art Stian Modified Trichrome in Cmd Siplas

Table 1 : Compared observer 1 and observer 2 with the modified technique

NUMBER SAMPLE	RESULT OBSERVER 1	RESULT OBSERVER 2	COMMENTS
250529	Cysts *Endolimax nana* ++	Cysts *Endolimax nana* ++	Agreement in identifying parasitic forms
252022	Cysts *Entamoeba coli* Cysts *Blastocystis Hominis*	Cysts *Entamoeba coli* Cysts *Blastocystis Hominis*	Agreement in identifying parasitic forms
252029	Yeasts ++	Yeasts ++	Agreement in identifying parasitic forms
253555	Cysts *Entamoeba coli* +	Cysts *Entamoeba coli* +	Agreement in identifying parasitic forms
253912	Cysts *Blastocystis Hominis*+	Cysts *Blastocystis Hominis*+	Agreement in identifying parasitic forms
255717	Cysts *Blastocystis Hominis*+	Cysts *Blastocystis Hominis*	Agreement in identifying parasitic forms
256920	Cysts *Endolimax nana*	Cysts *Endolimax nana*	Agreement in identifying parasitic forms
257583	Cysts *Endolimax nana* escasos	Cysts *Endolimax nana* +	Agreement in identifying parasitic forms
259110	Cysts *Blastocystis Hominis* +	Cysts *Blastocystis Hominis* +	Agreement in identifying parasitic forms
259209	Cysts *Blastocystis Hominis*++	Cysts *Blastocystis Hominis*++	Agreement in identifying parasitic forms
261161	Cysts *Blastocystis Hominis*++	Cysts *Blastocystis Hominis*+	Agreement in identifying parasitic forms
254021	Cysts Entamoeba coli ++	Cysts Entamoeba coli ++	Agreement in identifying parasitic forms
266114	Cysts Iodamoeba Butschlii+	Cysts *Iodamoeba Butschlii*+	Agreement in identifying parasitic forms
264223	Cysts *Endolimax nana* +	Cysts *Endolimax nana* + Cysts de *Blastocystis Hominis* escasos	Agreement in identifying parasitic forms
264532	Parasitic structuresare not observedin the sample	Parasitic structuresare not observed in the sample	Agreement in identifying parasitic forms

269688	Cysts Entamoeba hysto-litica/dispar ++	Cysts Entamoeba hysto-litica/dispar ++	Agreement in identifying parasitic forms
264746	Cysts *Endolimax nana* ++	Cysts *Endolimax nana* +	Agreement in identifying parasitic forms
264939	Leukocytes++	Leukocytes ++	Agreement in identifying parasitic forms
p- 03	Cysts Giardiaspp +	Cysts Giardiaspp +	Agreement in identifying parasitic forms

Observer 1: DAISSY VARGASS, Bacteriologyst CMDSIPLAS.
Observer 2: YENY BALLESTEROS,Bacteriologyst CMDSIPLAS.

II. Analysis of Results

a) Parasitic kappa index identification forms

		OBSERVER 1	
	-	concordance in identifying parasitic forms	negative
OBSERVADOR 2	concordance in identifying parasitic forms	16	0
	negative	0	1
	17	16	1
	%Sensitivity	100,0	
	%Specificity	100,0	

TABLE DE 2*2		
	Reference Reagent	No reference Reagent
Reagent to validate	Vp	Fp
No Reagent to validate	FN	VN
	Vp+FN	VN+Fp
Sensitivity	Vp/(Vp+FN)=True positives	
Specificity	VN/(VN+Fp)=True Negatives	

		Acceptability
ÍNDICE KAPPA	1,00	DIAGNOSTICACCURACYIS NOTED
VPP (%)	100,0	
VNP (%)	100,0	
Total positives	16	
Total Negatives	1	

ÍNDICE KAPPA	
Pe	0,886
Po	1,00

b) Kappa index leukocytes

	OBSERVER 1		
	-	concordance in identification	negative
OBSERVADOR 2	concordance in identifying parasitic forms	1	0
	negative	0	16
	17	1	16
	%Sensitivity	100,0	
	%Specificity	100,0	

TABLA DE 2*2		
	Reference Reagent	No reference Reagent
Reagent to validate	Vp	Fp
No Reagent to validate	FN	VN
	Vp+FN	VN+Fp
Sensitivity	Vp/(Vp+FN)=True positives	
Specificity	VN/(VN+Fp)=True Negatives	

	Aceptability
ÍNDICE KAPPA	1,00
VPP (%)	100,0
VNP (%)	100,0
Total positives	1
Total Negatives	16

ÍNDICE KAPPA	
Pe	0,003
Po	1,00

c) Kappa index yeast

	OBSERVER 1		
	-	concordance in identification in yeast	negative
OBSERVADOR 2	concordance in identifying parasitic forms	1	0
	negative	0	16
	17	1	16
	%Sensitivity	100,0	
	%Specificity	100,0	

TABLA DE 2*2		
	Reference Reagent	No reference Reagent
Reagent to validate	Vp	Fp
No Reagent to validate	FN	VN
	Vp+FN	VN+Fp
Sensitivity	Vp/(Vp+FN)=True positives	
Specificity	VN/(VN+Fp)=True Negatives	

	CRITERIO DE ACEPTABILIDAD	CRITERIO DE ACEPTABILIDAD
ÍNDICE KAPPA	**1,00**	DIAGNOSTICACCURACYIS NOTED
VPP (%)	100,0	
VNP (%)	100,0	
Total Positives	1	
Total Negatives	16	

ÍNDICE KAPPA	
Pe	0,003
Po	1,00

Kappa: the agreement between observers for the identification of parasitic forms, leukocytes, yeasts, and negative for themis 1.0, which shows diagnostic accuracy and level of agreement between observers for the samples with the latest changes made by SIPLAS medical laboratory, concluding that the changes mentioned here allow adequate identification of both parasite forms leukocytes, yeast and other fungal forms structures that allow the definition diagnosed patients, ensuring diagnostic accuracy versus the clinical definition

kappa	Degree of agreement
< 0	without agreement
0 – 0.2	insignificant
0.2 – 0.4	low
0.4 – 0.6	moderate
0.6 – 0.8	good
0.8 – 1	very good

III. Sensitivity and Specificity

The sensitivity and specificity of the samples analyzed for fungal structures, yeast and parasiticl-eucoidesis 100%, which shows that the stain can classify patients according to the irpositive or negative real state against it sclinical definition

a) Parasitic forms identification with modified technique

Micrographs of amoebae obtained modified tech-nique implemented

Figure 1 : Cysts Blastocystis hominis

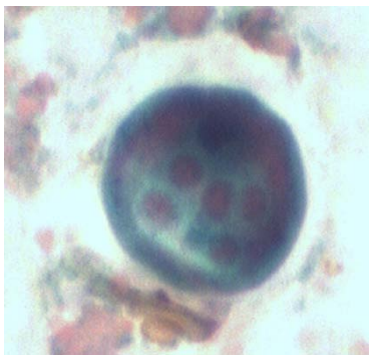

Figure 2 : Cysts Entamoeba coli

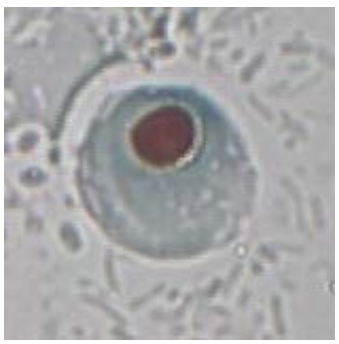

Figure 3 : Cysts Iodamoeba butschlii

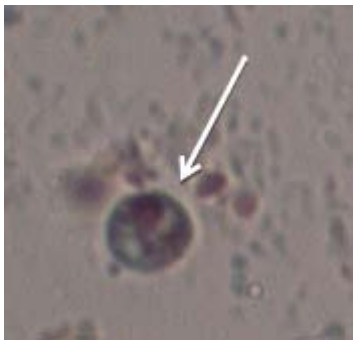

Figure 4 : Cysts Endolimax nana

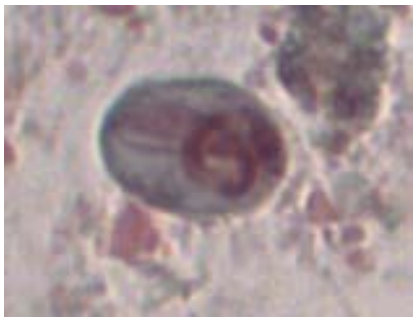

Figure 5 : Cysts Giardia Duodenalis

Note: The photomicrographs were taken by the bacteriologys Daissy Vargas Sepulveda in CMD SIPLAS

IV. Conclusions

- The quick method is effective and accurate.
- It requires less processing time and therefore the patient must wait less time to get your results
- Iteliminates contamination of the reagents considering that it is not necessary to use Koplic
- It saves time and money by having as only three reagent required to implement this technique

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