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TOXIC EFFECT OF AMPHOTERICIN B ON THE SPERM COUNT IN SWISS ALBINO MICE

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Abstract

The anti-leishmanial drug Amphotericin B is widely used in the treatment of leishmaniasis. Although its toxic effects on testis is causing abnormalities in spermatogenesis has not been studied. Hence the present study has been conducted to evaluate the effect if Amp B on epididymal sperm counts. Male mice were employed in the study (n = 5per group). The animals were administered (i.p) with dose of Amphotericin B (6 mg/kg body weight) for 30 days and the vehicle and control treated with glucose and untreated respectively. Samples were obtained after 24 h, 2 week, 4 weeks and 6 weeks of the treatment. Mice were sacrificed and epididymis was collected in 2 ml of normal saline, minced filtered and stain with Nigrosin and Eosin y. The sample was taken in leucocyte pipette, diluted with sperm diluting solution and sperm count was done as per the procedure of WHO. The result of this study reveals that Amphotericin B shows significant decrease in the sperm count in a dose and time dependent manner.

Introduction

Amphotericin B has been effectively used in the treatment of Leishmaniasis. It was known to induce nephrotoxicity, cardiotoxicity but its effect on reproductive toxicity is not yet known. But a spermicidal effect is known as a contraceptive in bull and rabbits.(Robert H. Foote). However it is not known whether Amphotericin B has any effect on the number of spermatozoa as sperm concentration. As Amphotericin B is delivered to all age group of persons, hence this study was undertaken to investigate the effect of Amphotericin B on sperm count.

Material and Methods

Male Swiss Albino Mice strain weighing (26 -36 gm) (11 -12 week old) were maintained under standard laboratory condition in polypropylene cages. They were fed with pelleted food and water ad libitum. Five animals were chosen for each group. The doses of Amphotericin b were freshly prepared 6 mg/kg body weight and injected (i.p) for 30 days at a time interval of 24 h and the control group received glucose and untreated. Animals were sacrificed by using anaesthesia on the 24 h, 2 week, 4 week and 6 week of the last treatment and the epididymis were collected and minced in 2 ml of normal saline (p^H 7.2). The suspensions free from debris were mixed with Eosin Y and Nigrosin (WHO 2010). 0 .67 gm of Eosin Y dissolves in 100 ml of 0.9% of NaCl with gentle heat and 10 % of Nigrosin dissolve to 100 ml of Eosin y solution. Both the solution were boiled and then allowed to cool and filter through filter paper and store in sealed dark bottle for use. All the reagents were freshly prepared. Now the aliquot of stained the filtrate was taken in WBC pipette upto the mark of 0.5 and diluted further up to mark 11 through sperm diluting solution. The mixture was shaken and dropped into Neubauer's Chamber and kept in humid chamber for 45 minutes and then sperm count was performed. The sperm count in 5 corner of Small Square of 1 mm each

area and multiplied by 5×10^4 factor to calculate total number of sperm. Data were analysed by ANOVA Test using SPSS Software and P< 0.001, was considered as a level of significant.

Result

A significant decrease in the sperm count was noted in the epididymal suspension, on receiving 6 mg/kg body weight of Amphotericin B injection. The highest fall in the sperm count was recorded in the sample collected after 24 h and 2 week of the treatment of Amphotericin B. The decrease in sperm count was significantly compared with control and vehicle treated group (P<0.001). The 4th week and 6th week sample does not shows such a reduce sperm count. There was a dose dependant decrease in the number of spermatozoa, which further reveal to normal as a time progress.

Discussion

Clinical use of Amphotericin B was limited due to number of factors. The bioavability of Amp B in organ tissue is small as the drug extensively binds to cholesterol containing cell membrane. Amphotericin B is known to be highly protein bound molecule which never the less rapidly leave the circulation. In the present study a drastic decrease in the sperm count was observed in the animal treated with Amp B for 30 days. Decrease in sperm count strongly determines the spermatotoxic effect of Amphotericin B. The similar effect was observed in the mice treated with food prepared in microwaves. Statistical analysis of the mean value which shows significance value (P<0.001). Odeigah (1997) reported that exposure to the chemical could produce pituitary –hypothalamic and sex hormonal effect which in turn could affect spermatogenesis or exposure could cause abnormalities in structural and functional impairment of sperm.

SPERM CONCENTRATION

	UTC	VTIC	24 H	2 W	4 W	6 W	VTTC
MICE 1	4900000	4550000	1150000	1700000	4100000	2550000	4400000
MICE 2	5150000	2550000	450000	550000	5150000	5500000	1350000
MICE 3	4300000	3000000	550000	950000	1900000	5650000	750000
MICE 4	6450000	6300000	1300000	1200000	1500000	5600000	1900000
MICE 5	6300000	2600000	650000	750000	4100000	4050000	1300000
SUM	27100000	19000000	4100000	5150000	16750000	23350000	9700000
AVG	5420000	3800000	820000	1030000	3350000	4670000	1940000

Tests of Between-Subjects Effects

Dependent Variable:Value

Source		Type III Sum of Squares	df	Mean Square	F	Sig.
Intercept	Hypothesis	3.159E14	1	3.159E14	268.501	.000
	Error	4.706E12	4	1.177E12[a]		
Treatment	Hypothesis	9.582E13	6	1.597E13	10.628	.001
	Error	3.606E13	24	1.503E12[b]		
Variable	Hypothesis	4.706E12	4	1.177E12	.783	.547
	Error	3.606E13	24	1.503E12[b]		
Treatment * Variable	Hypothesis	3.606E13	24	1.503E12	.	.
	Error	.000	0	.[c]		

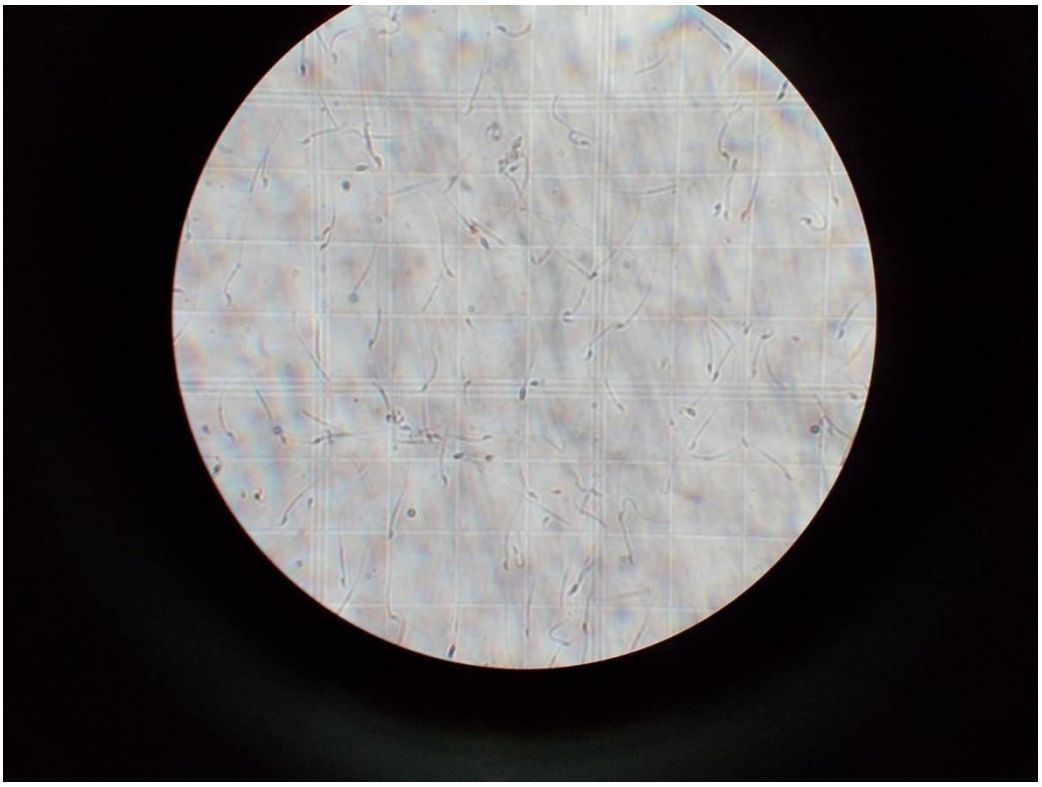
a. MS(Variable)

b. MS(Treatment * Variable)

c. MS(Error)

Sperm concentration

UTC



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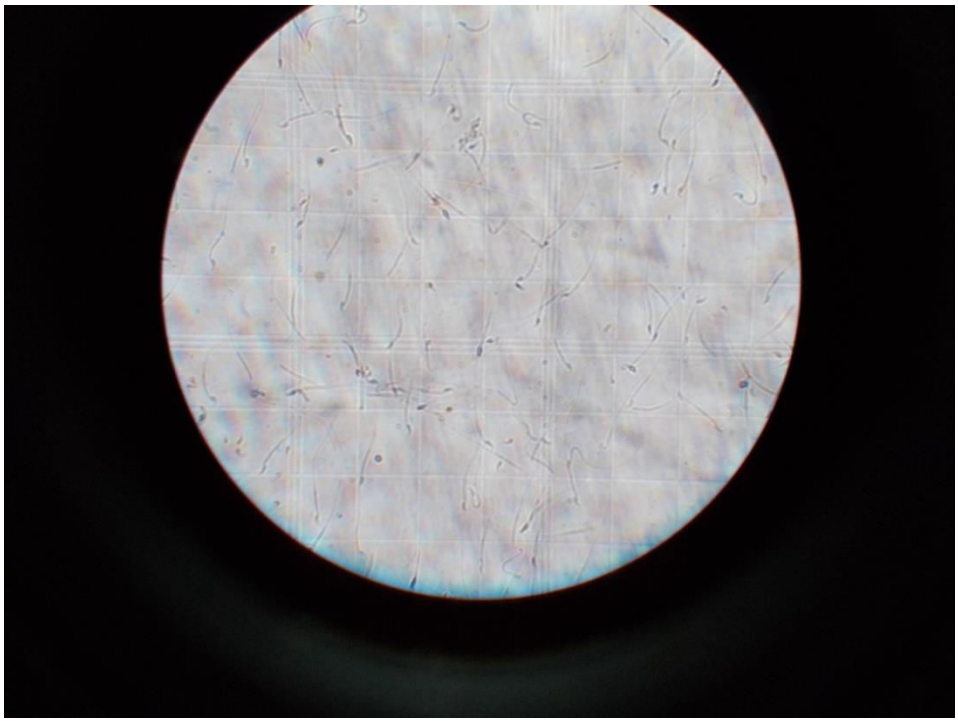

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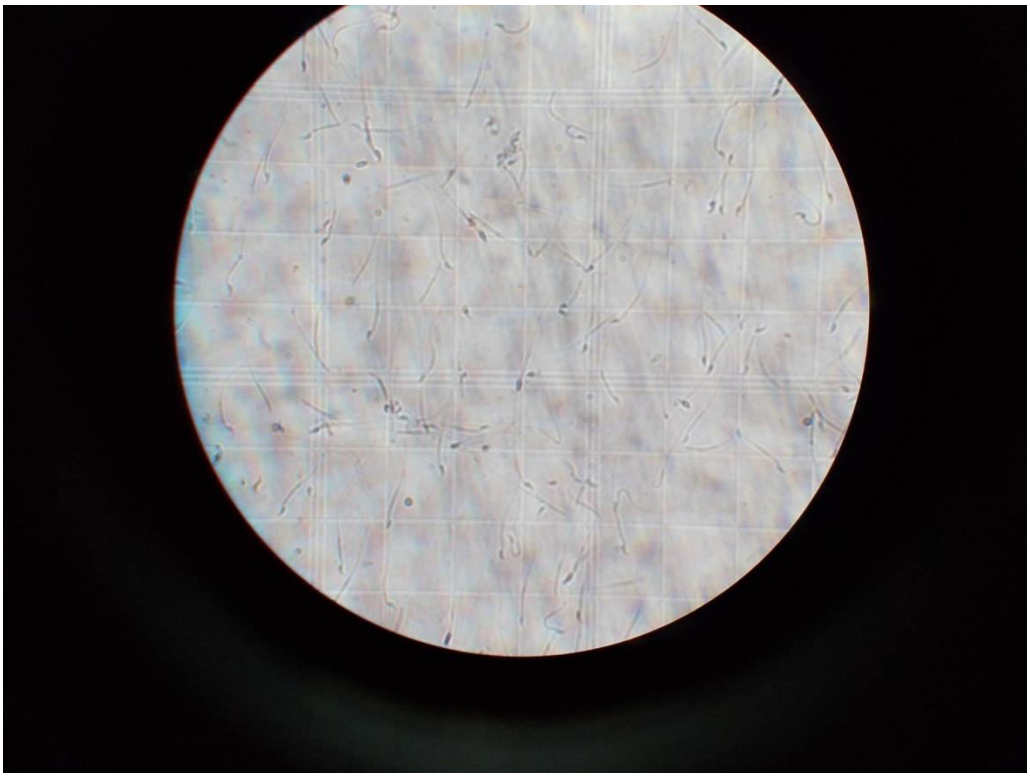

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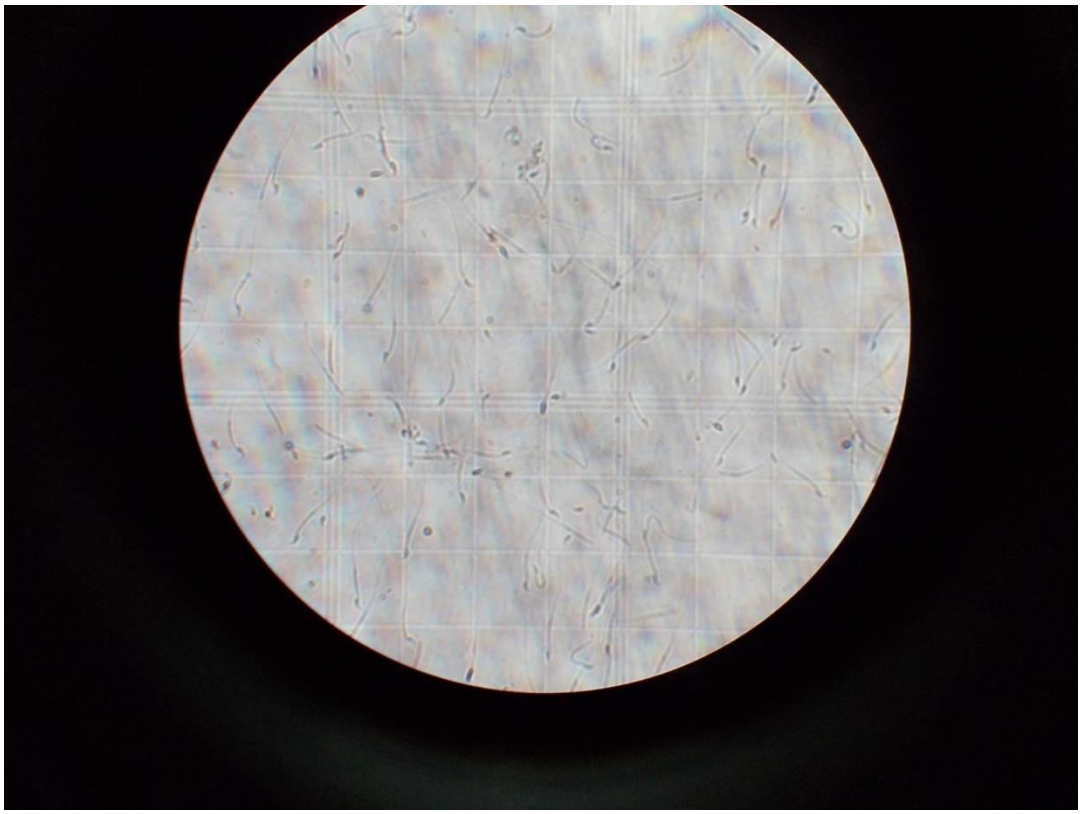

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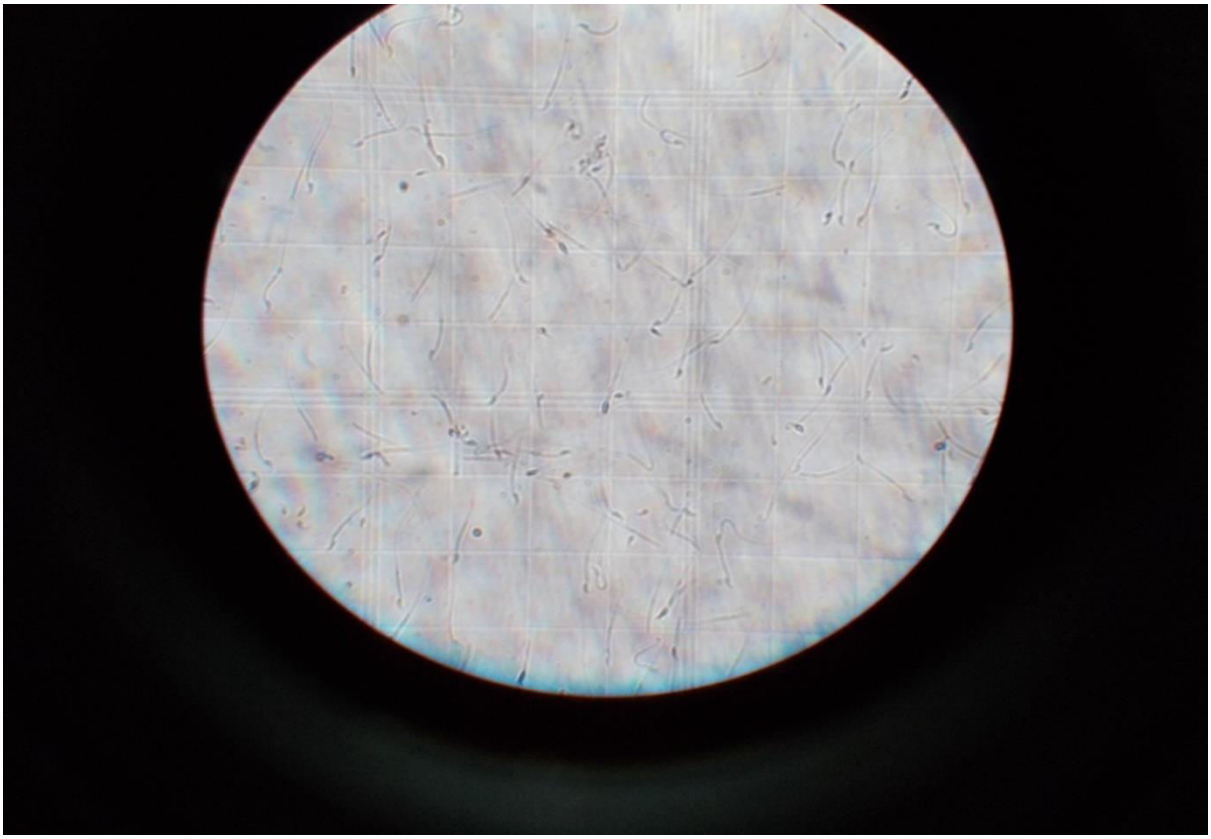

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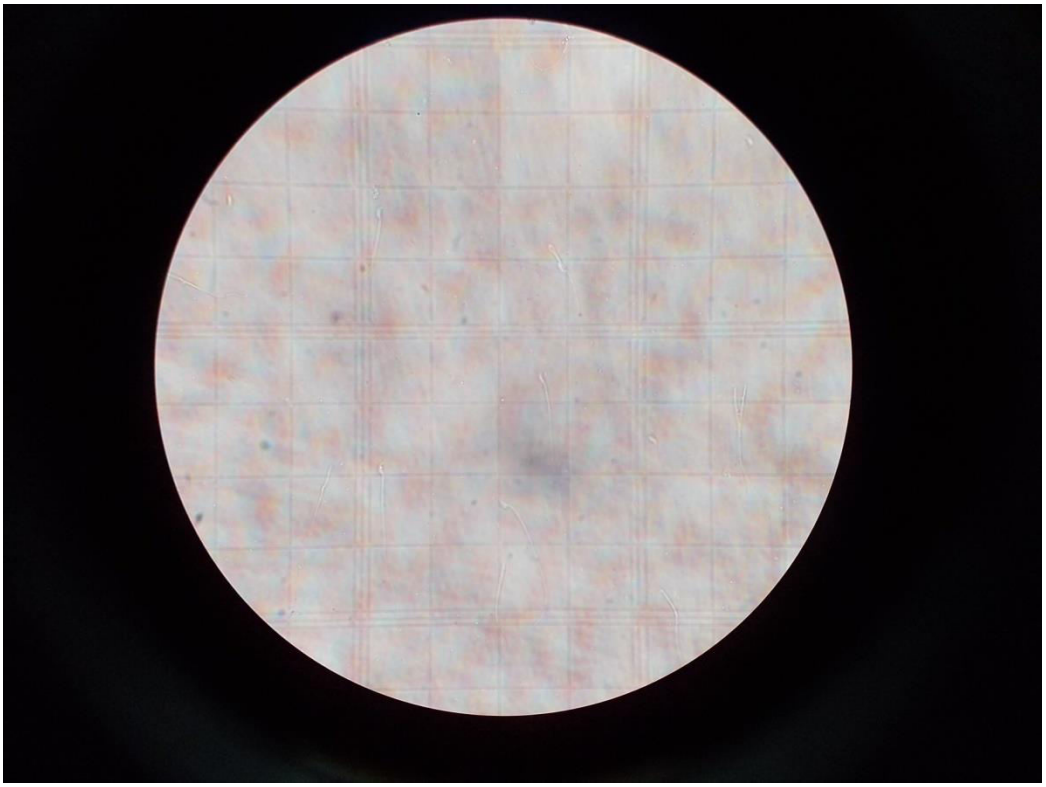

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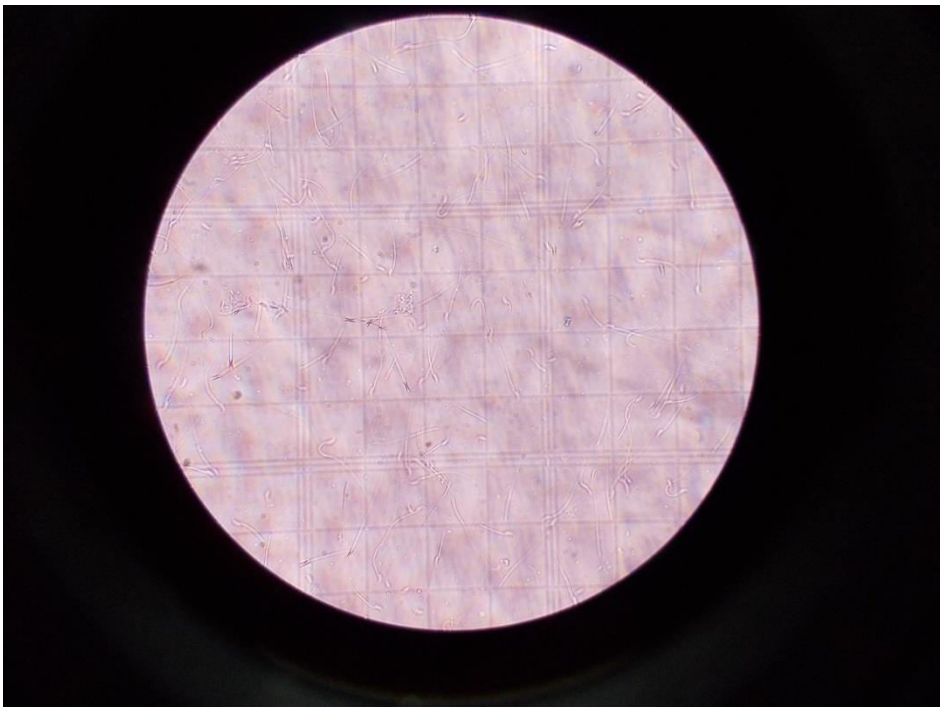

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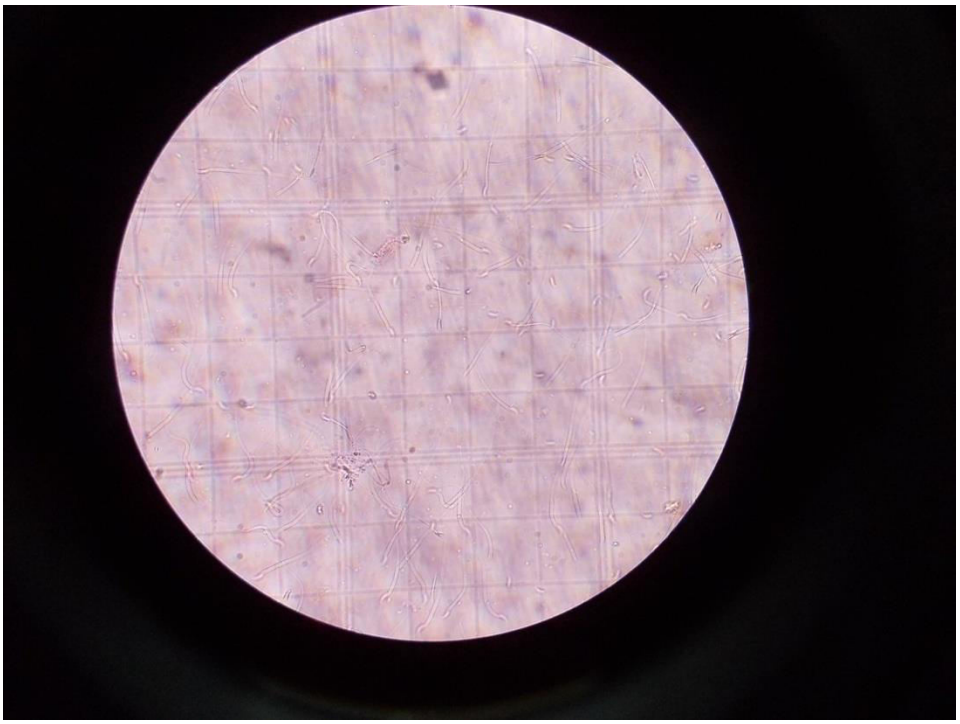

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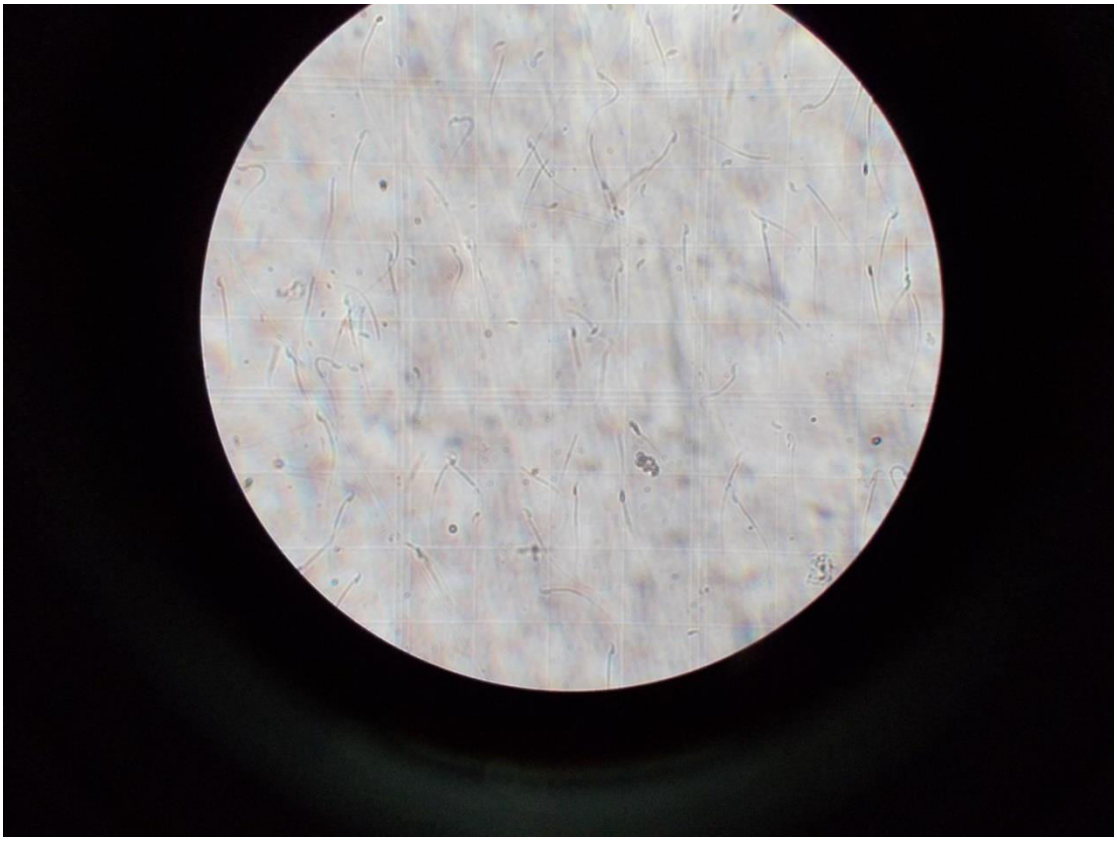

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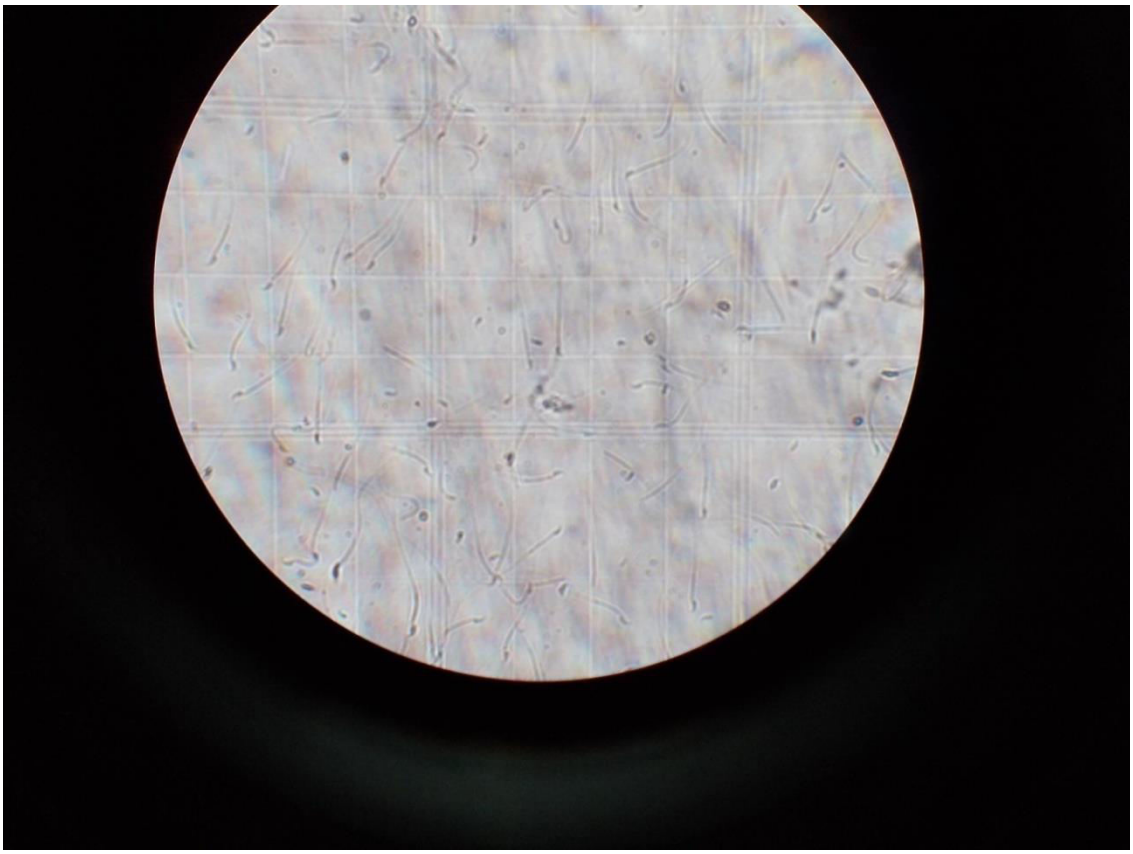

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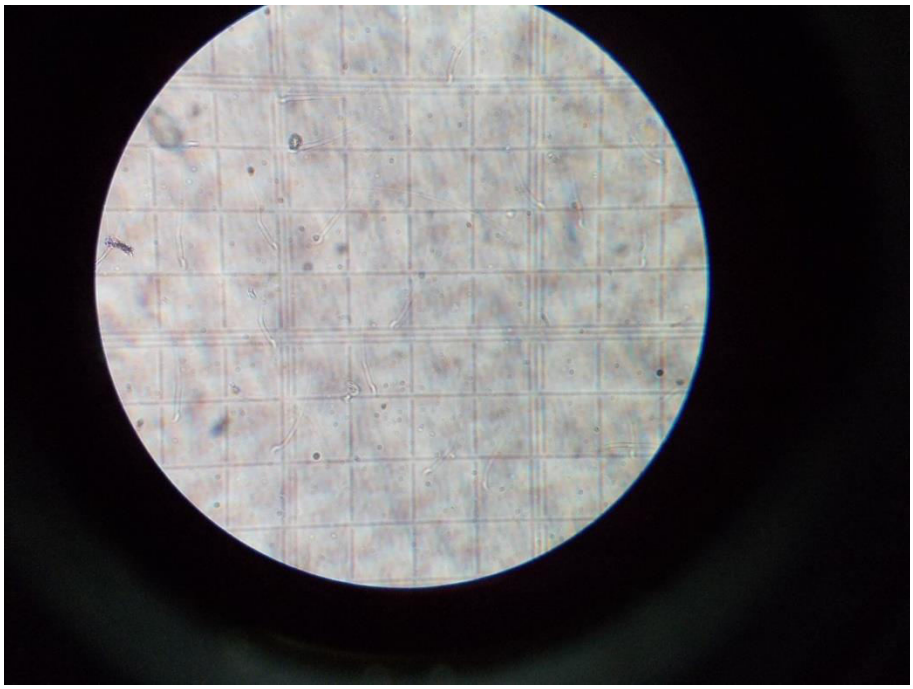

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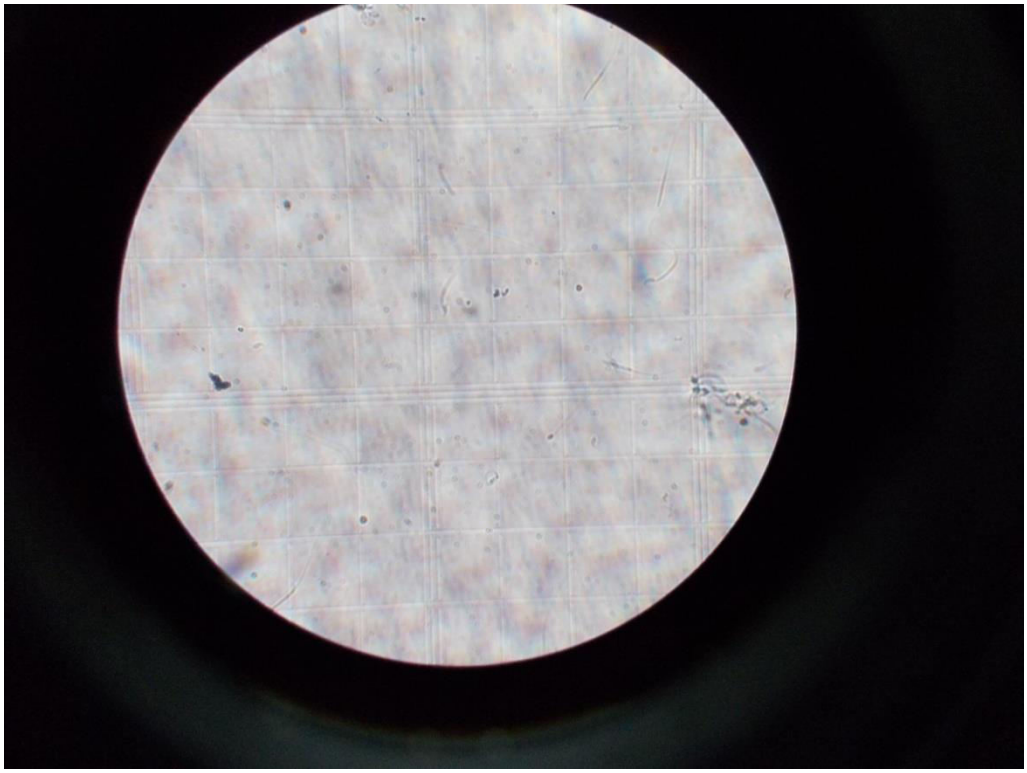

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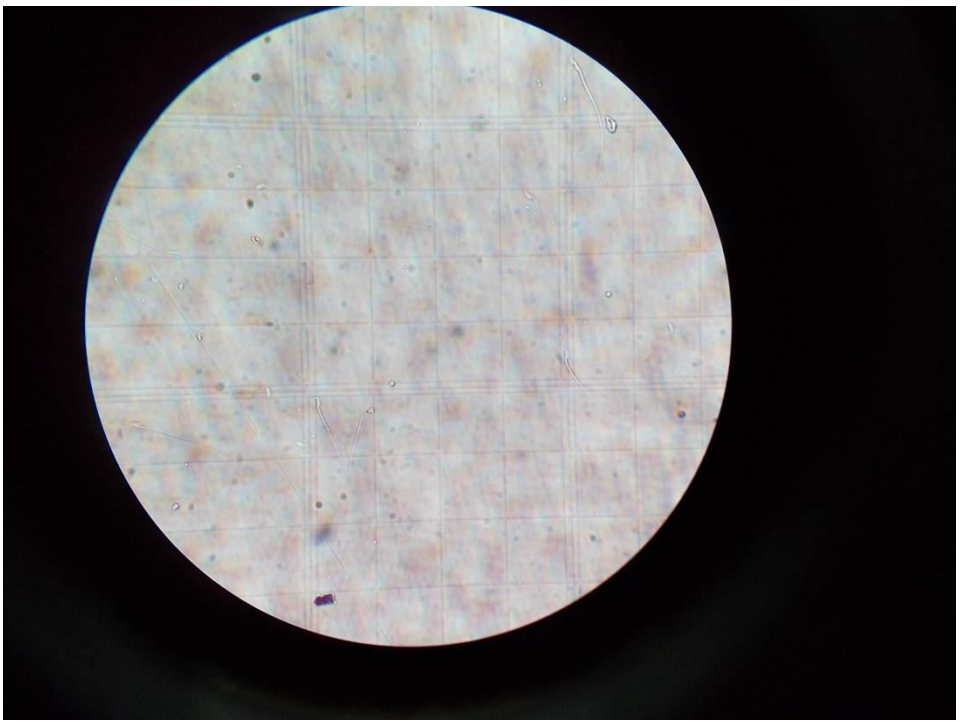

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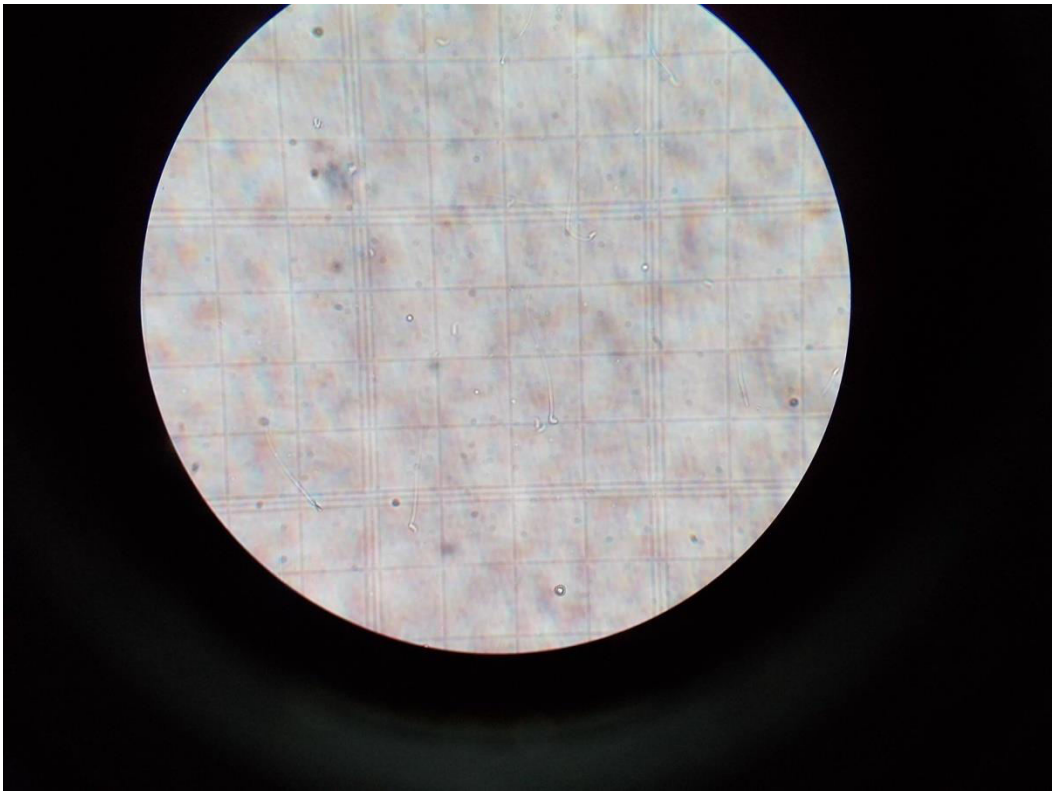

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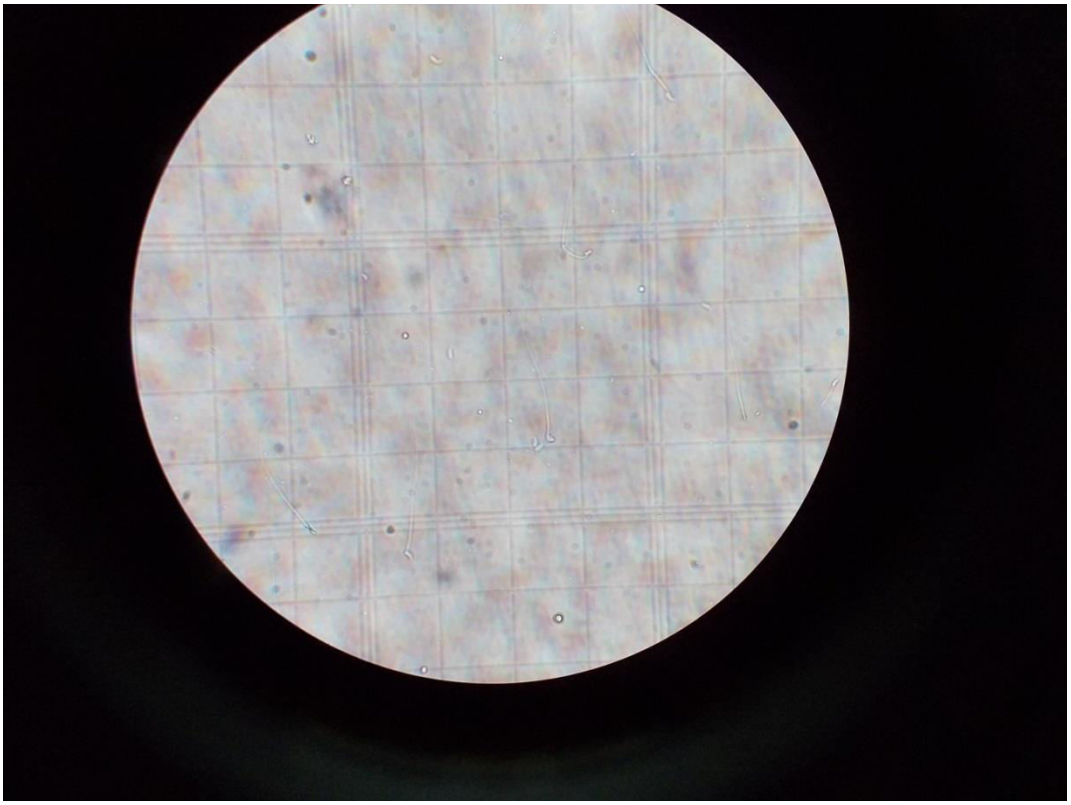

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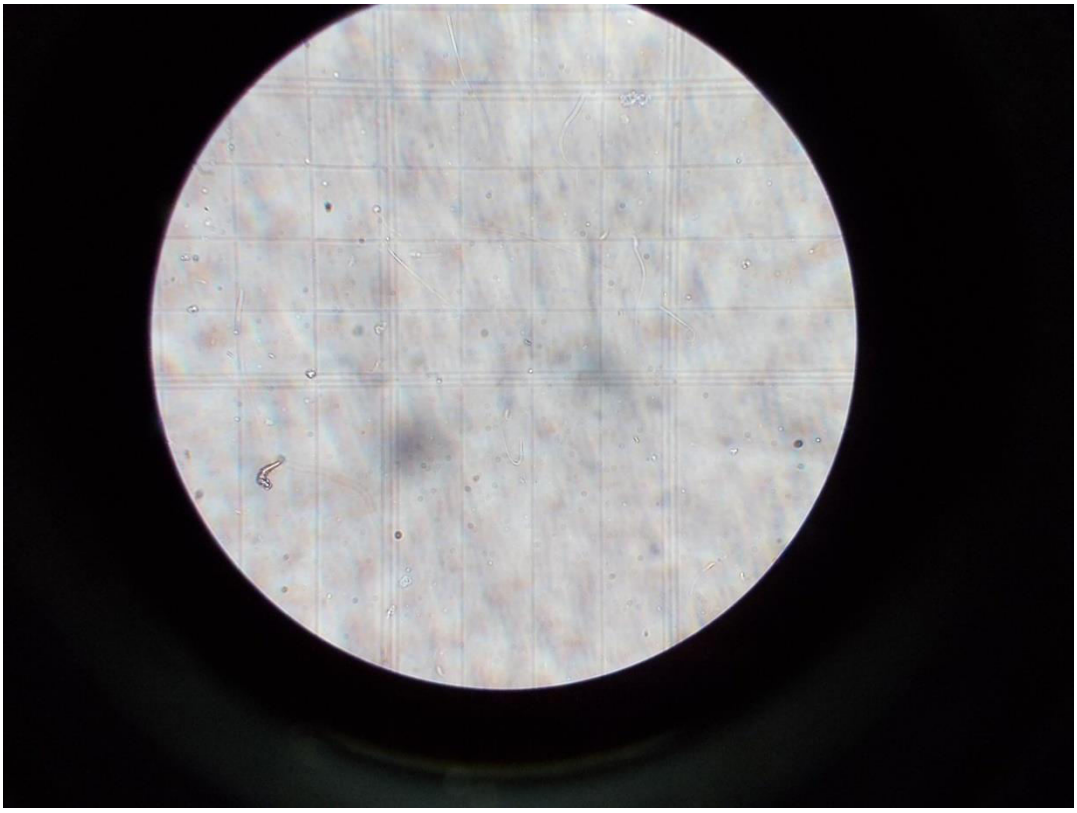

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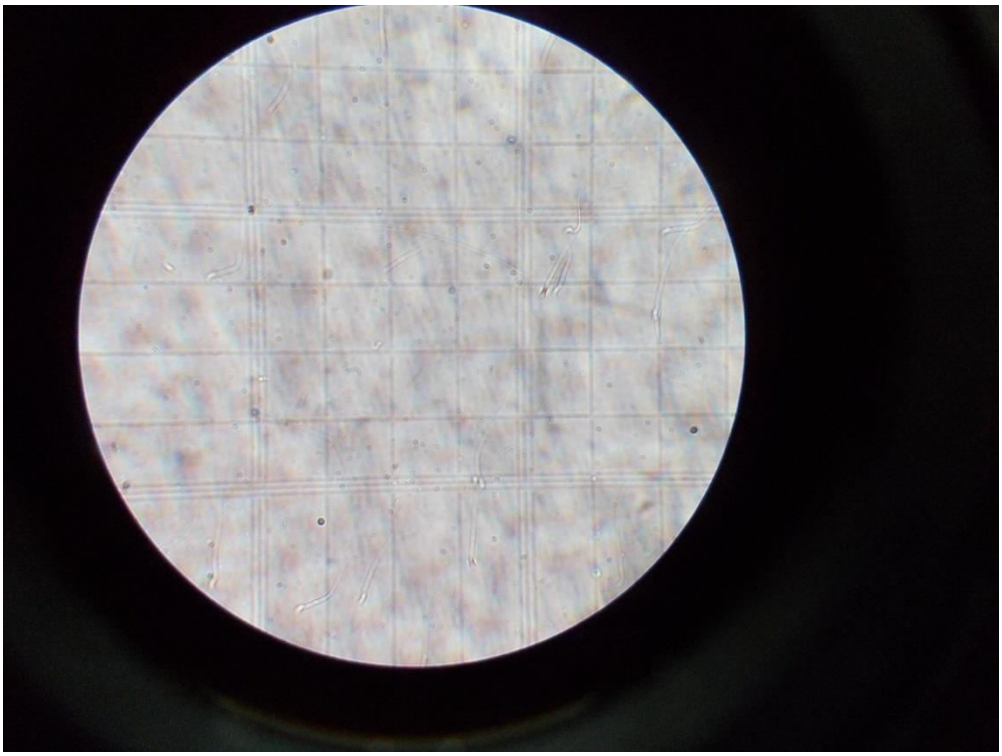

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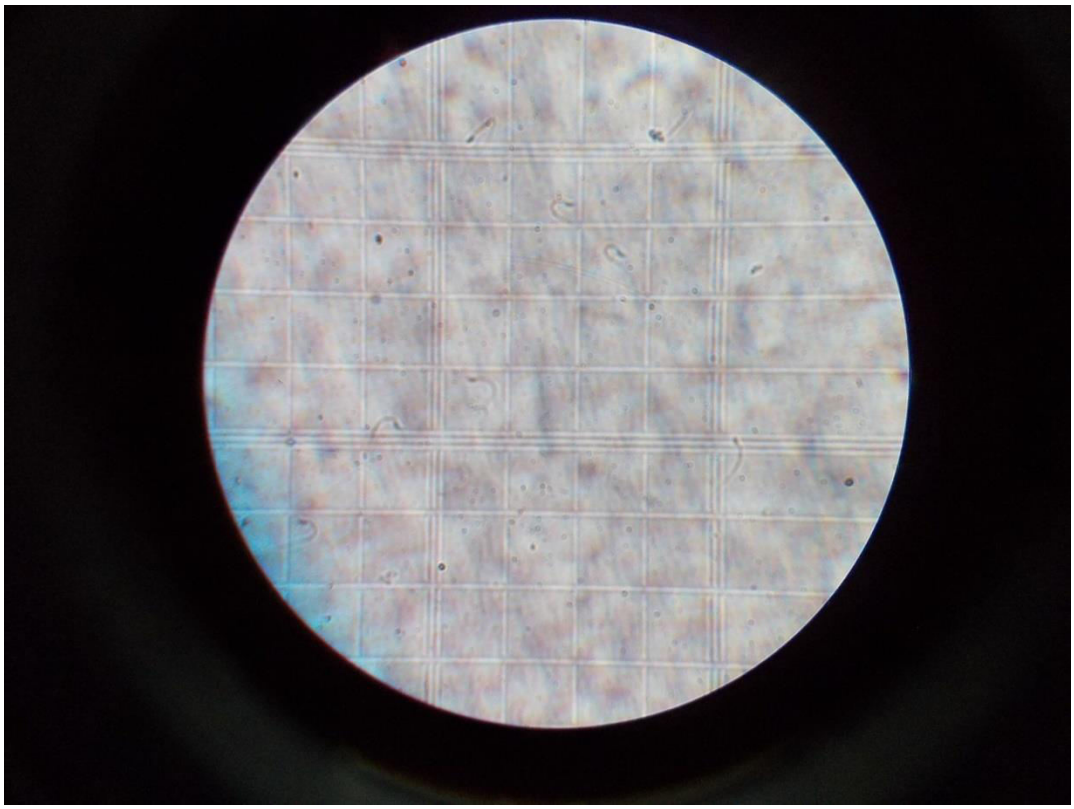

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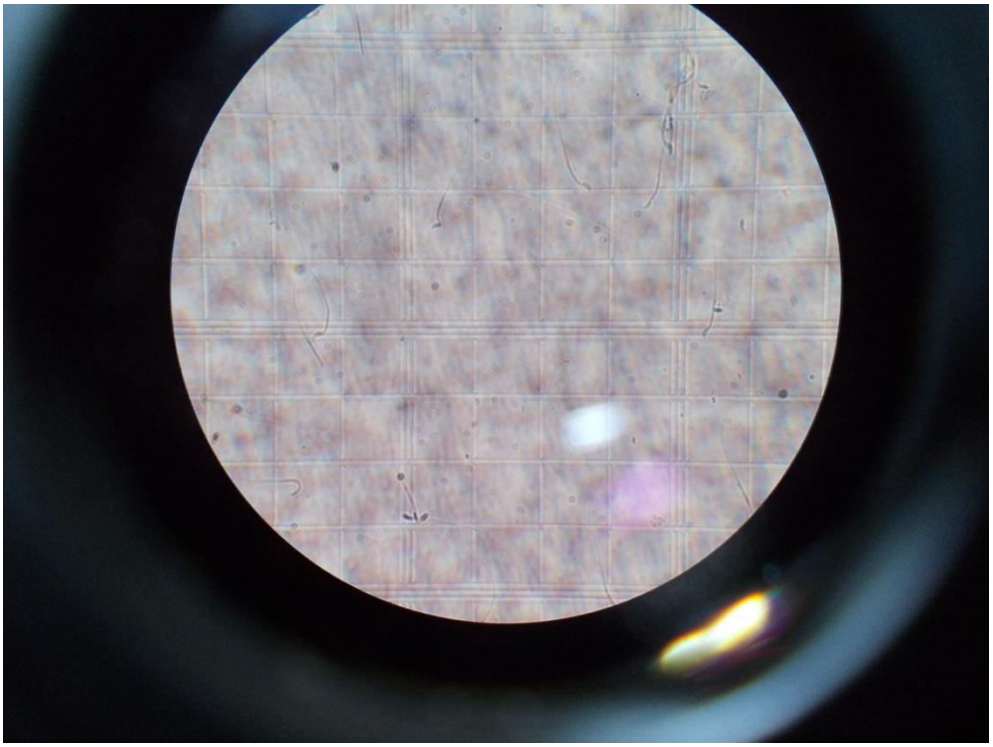

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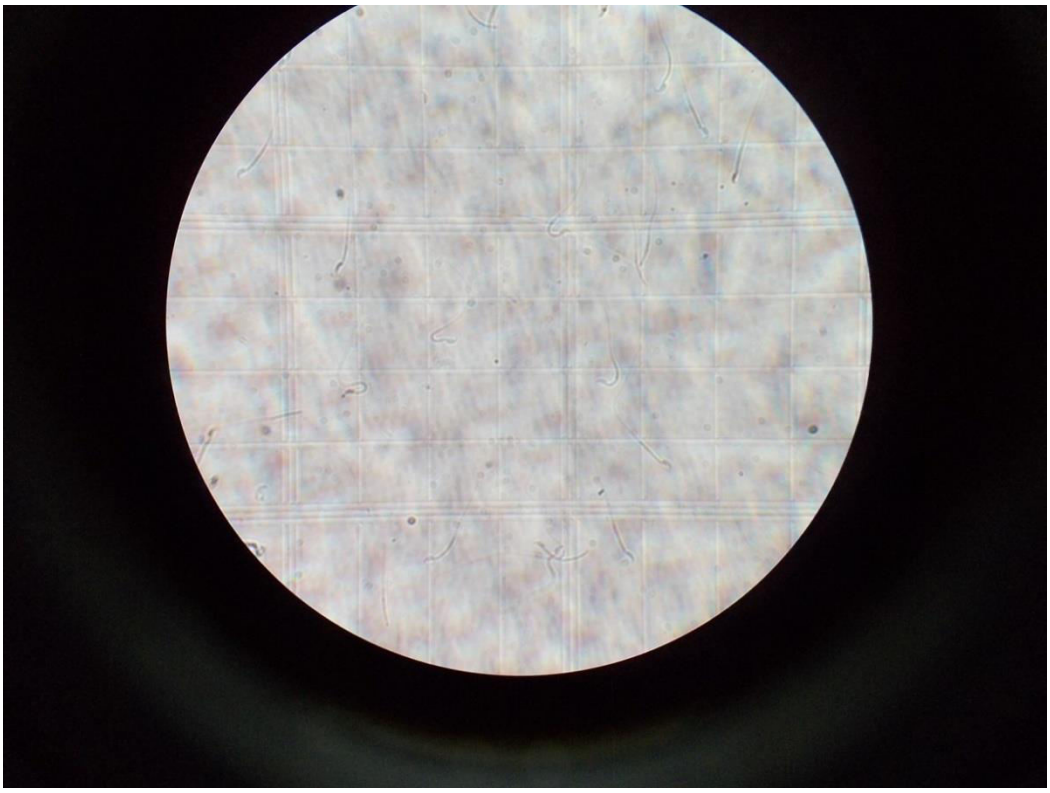

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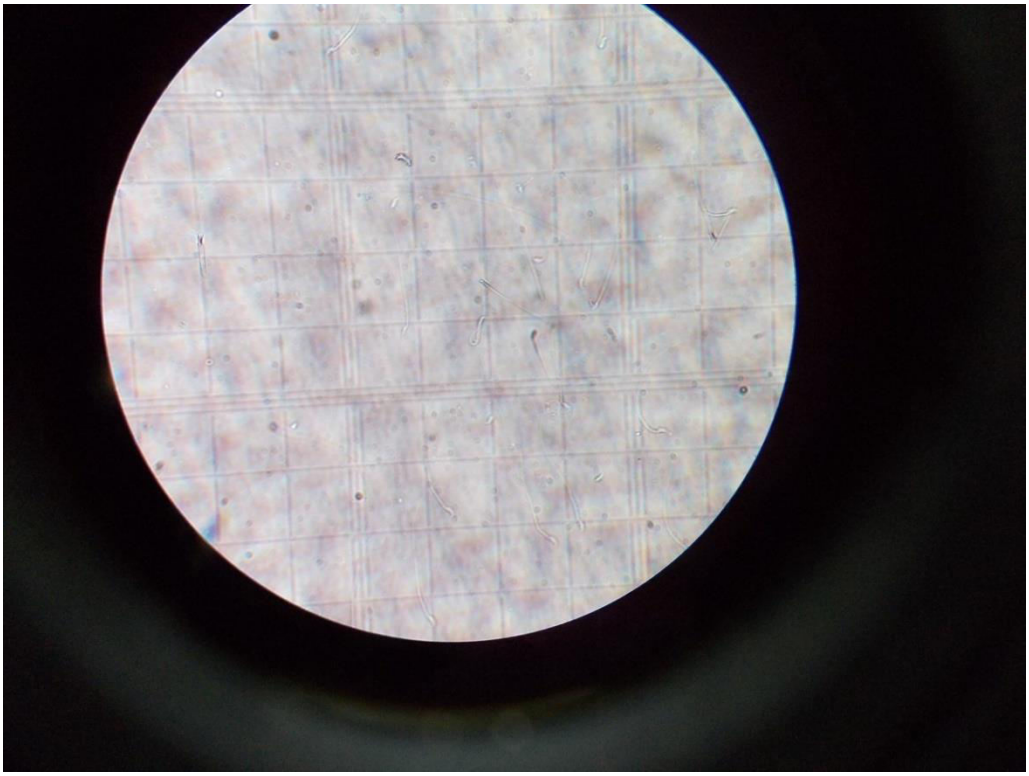

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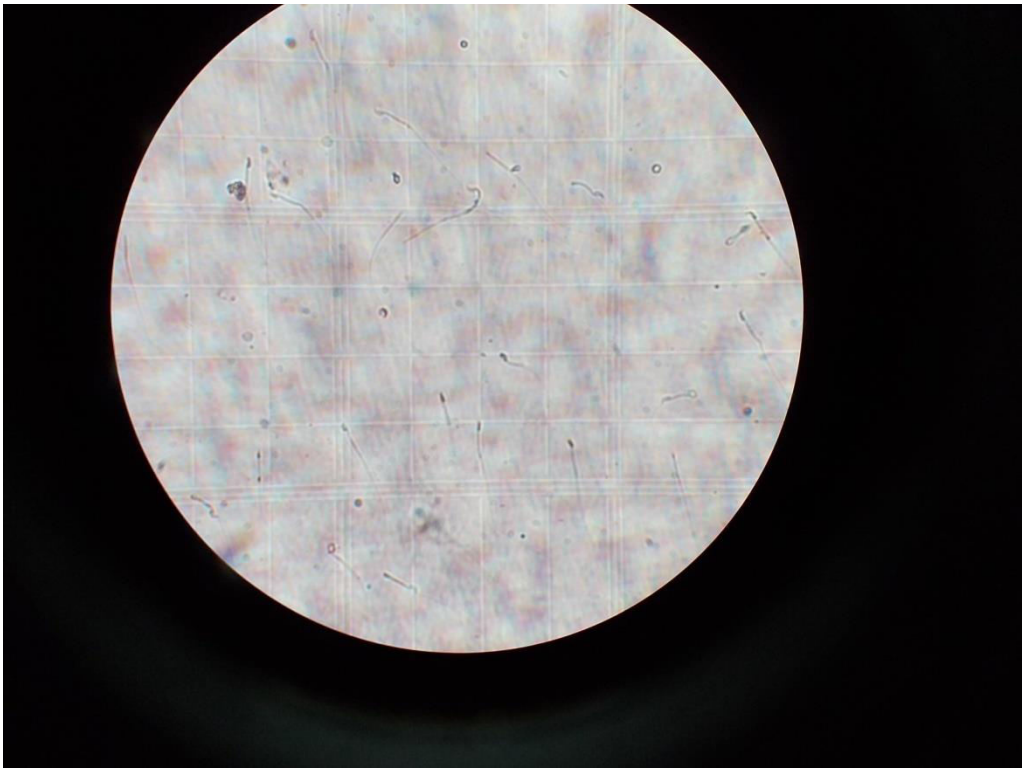

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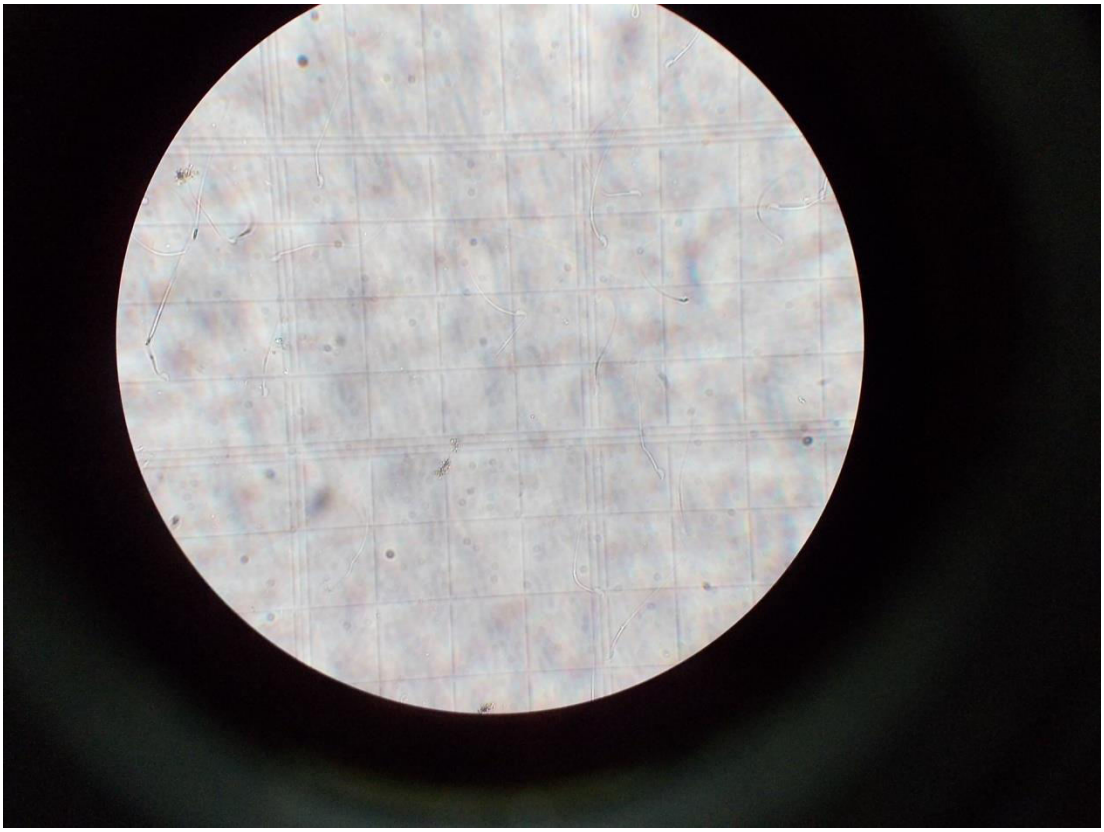

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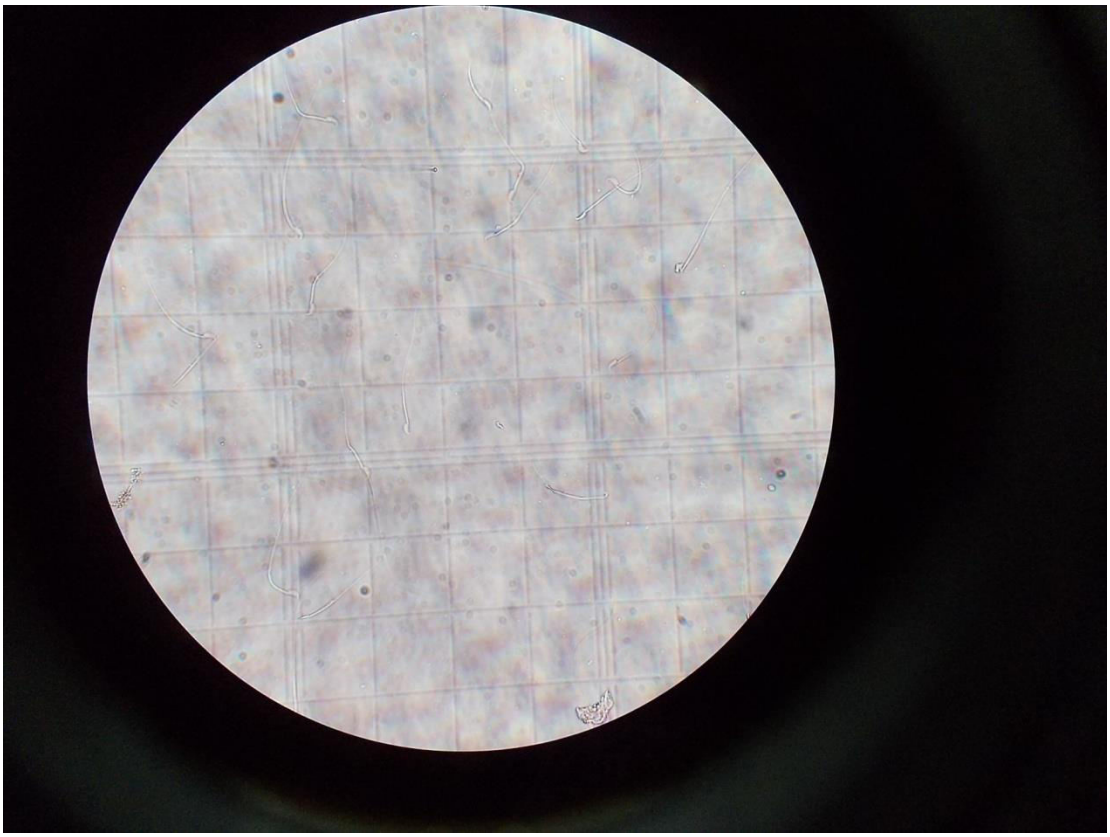

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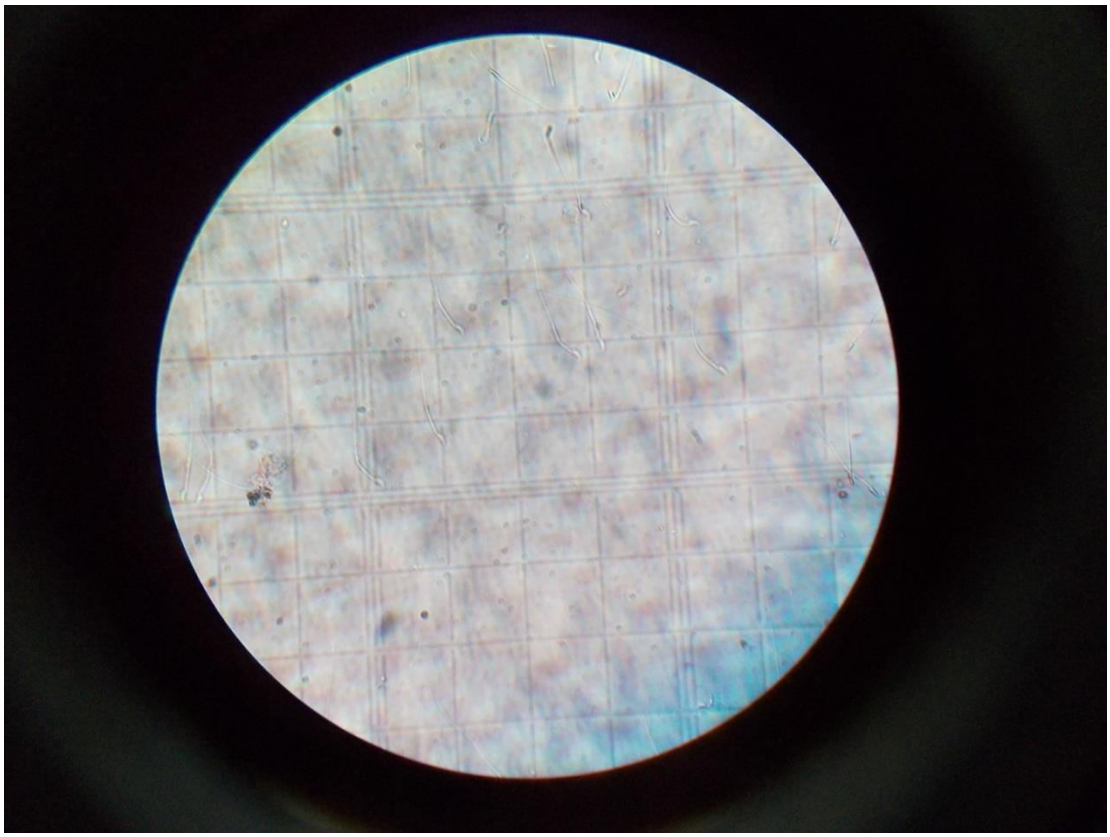

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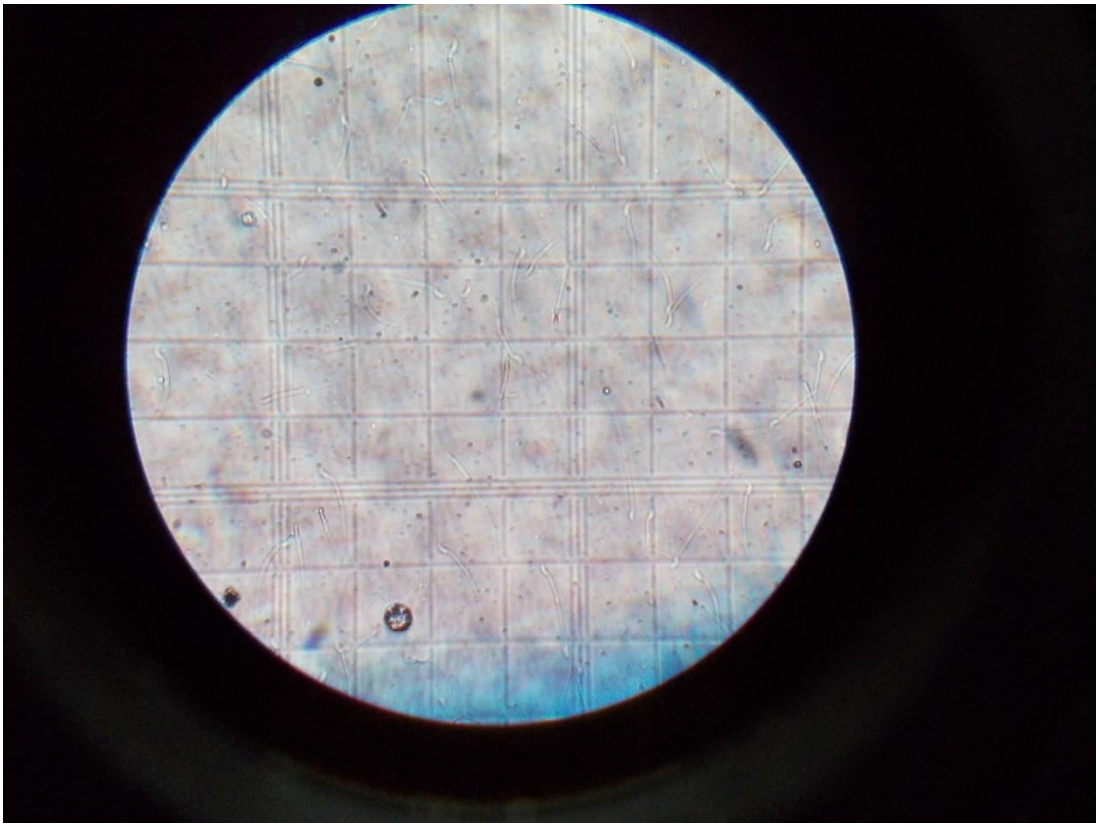

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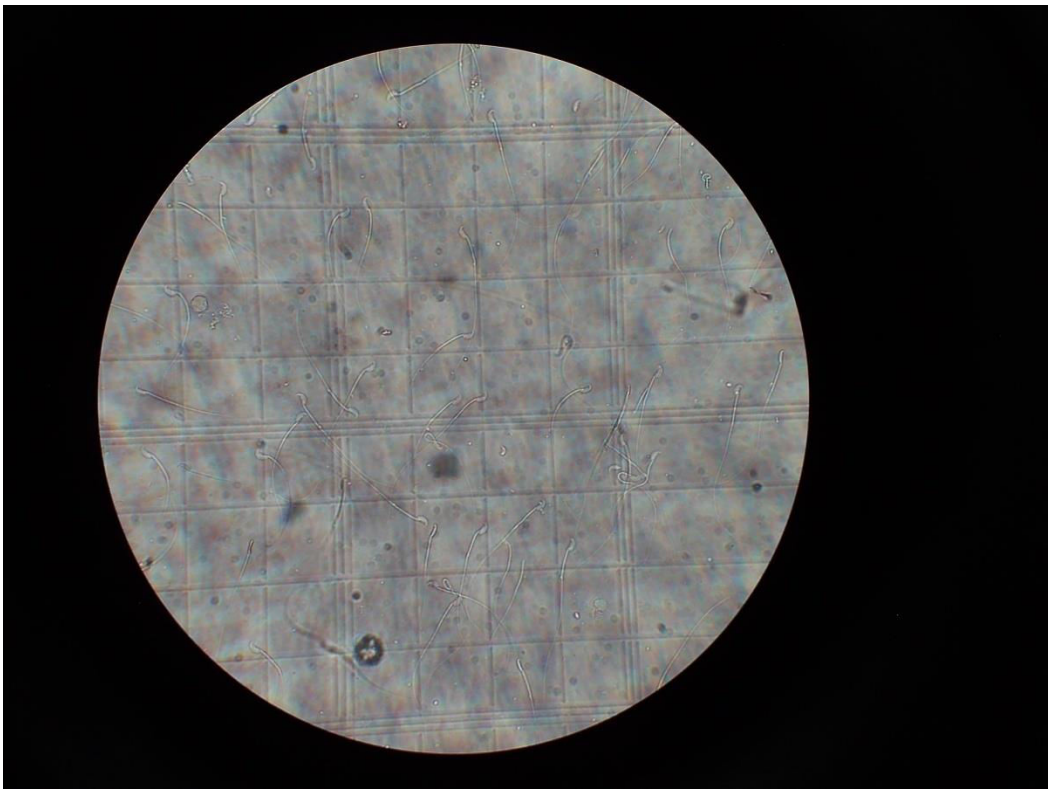

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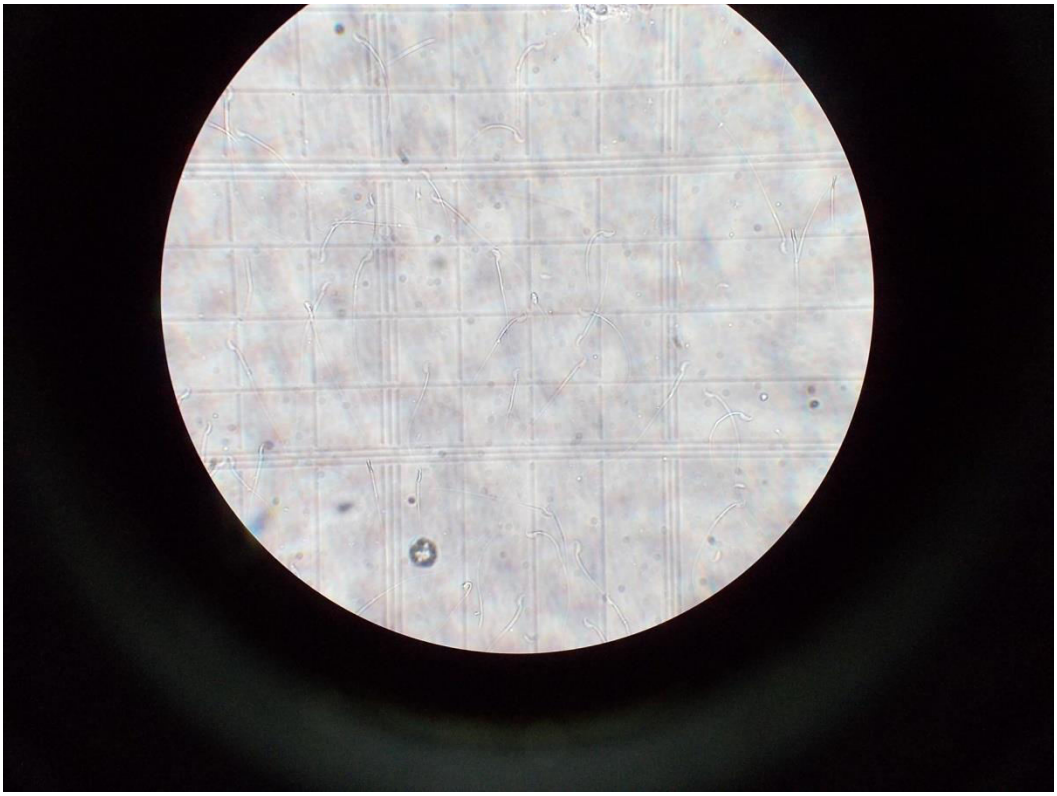

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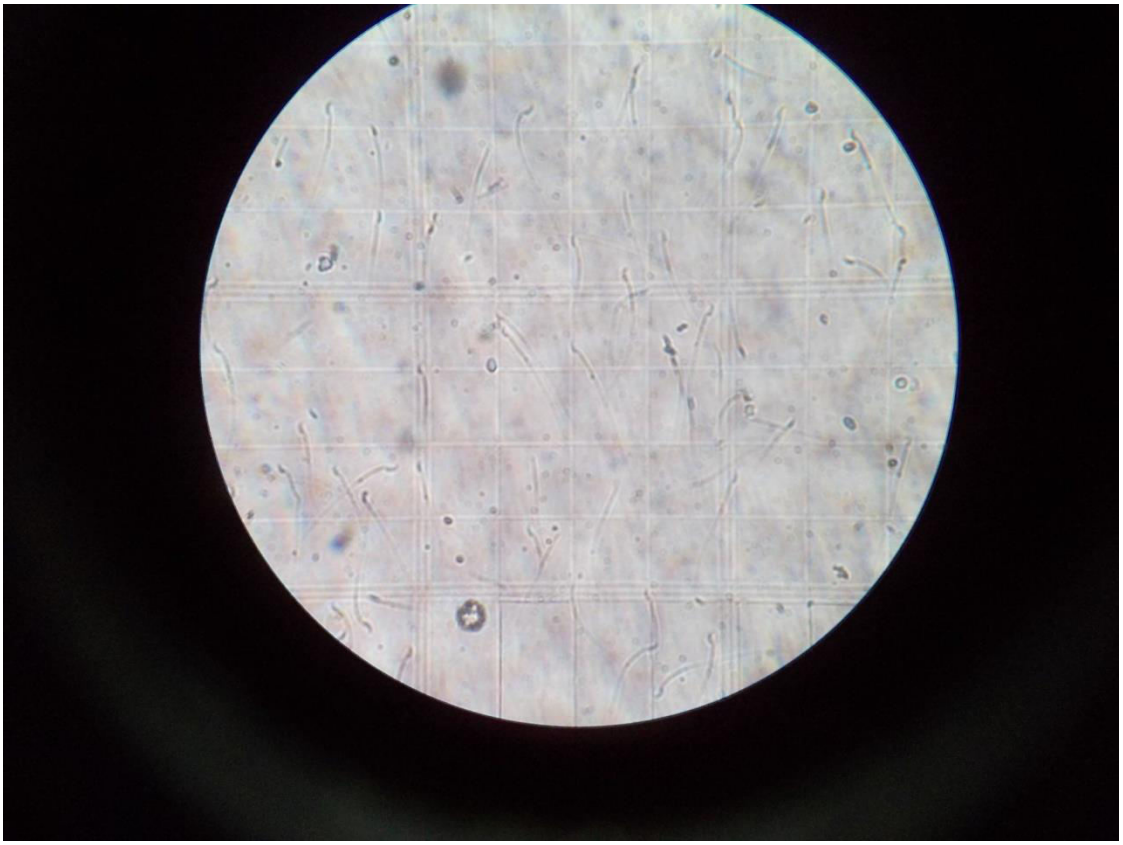

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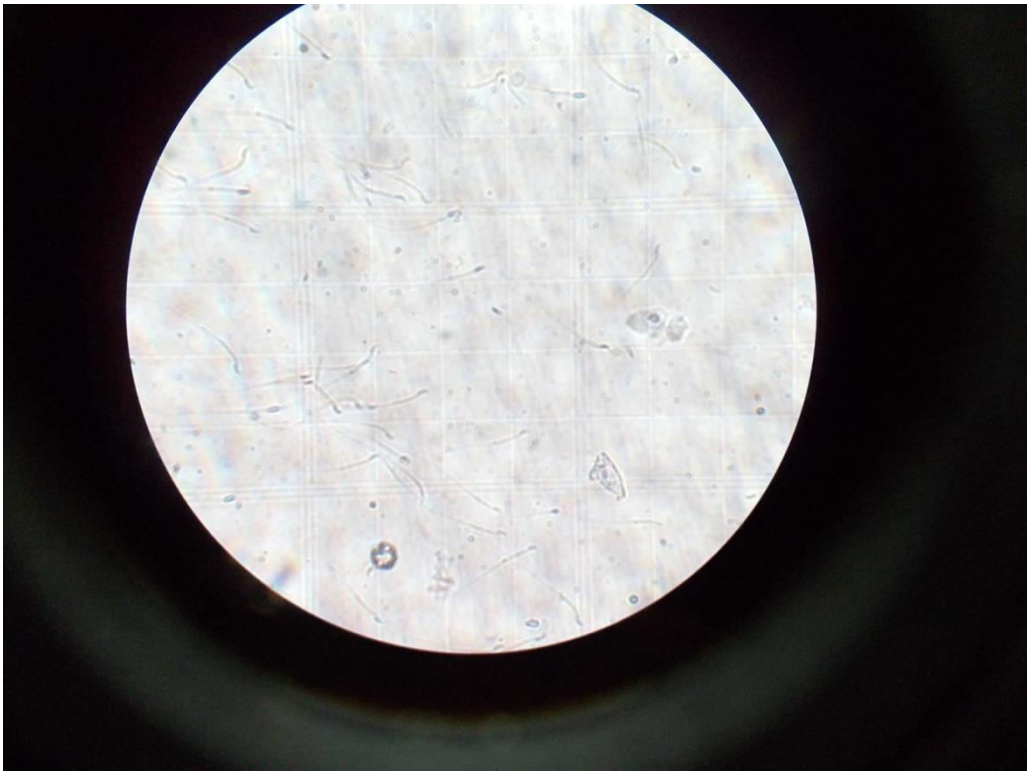

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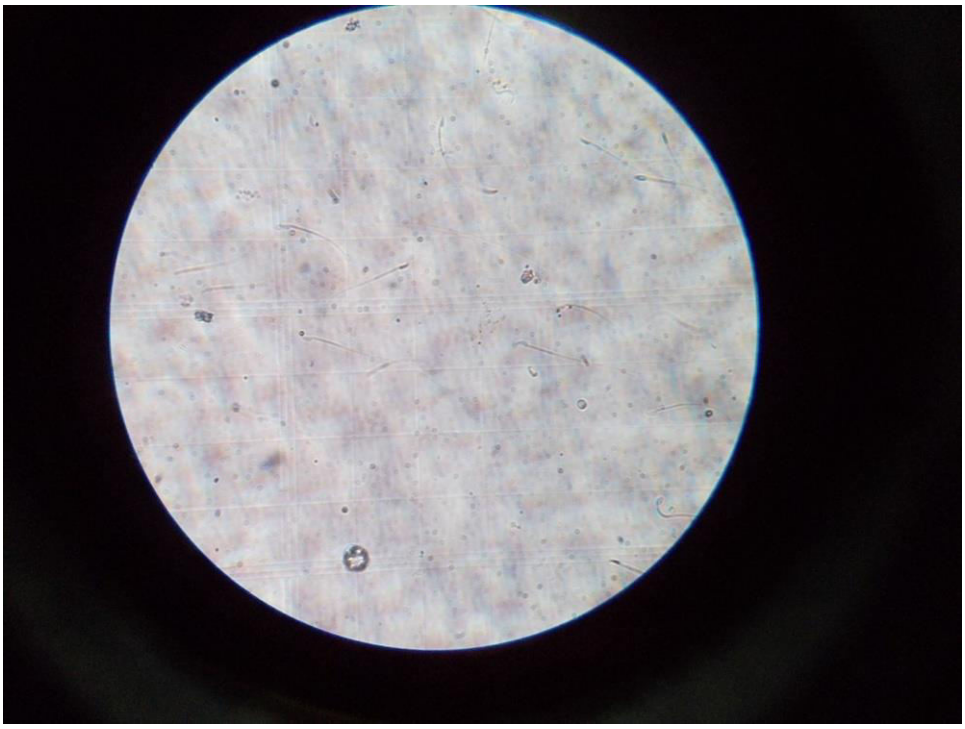

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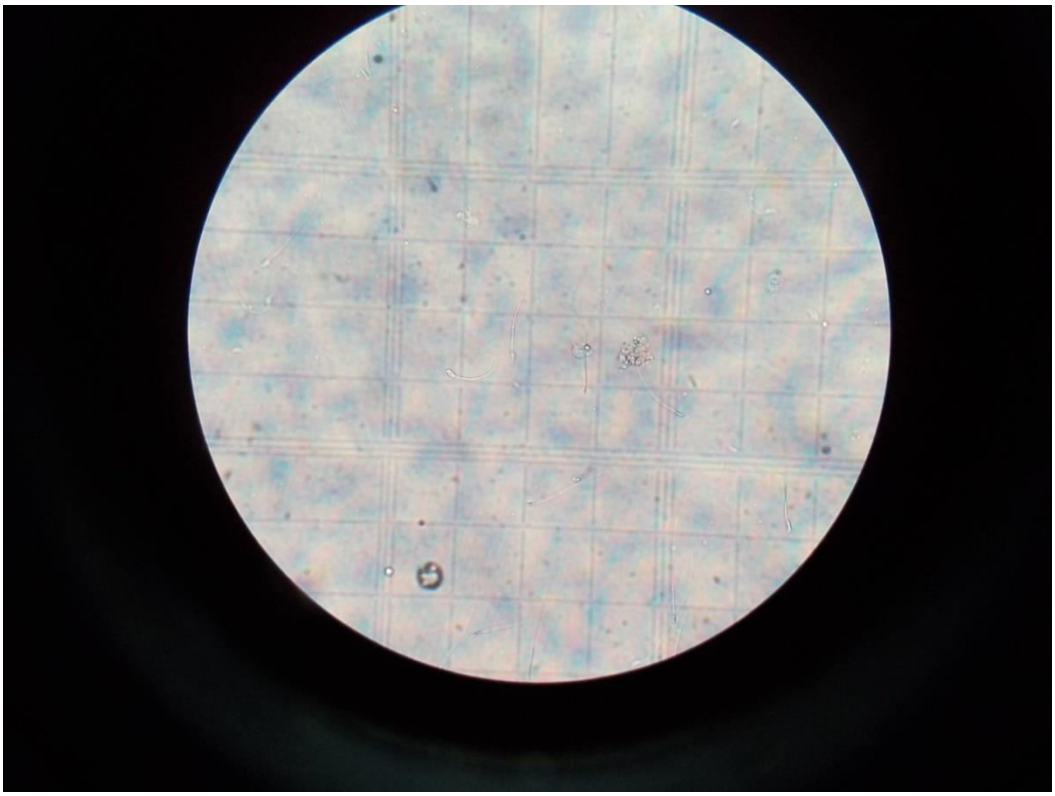

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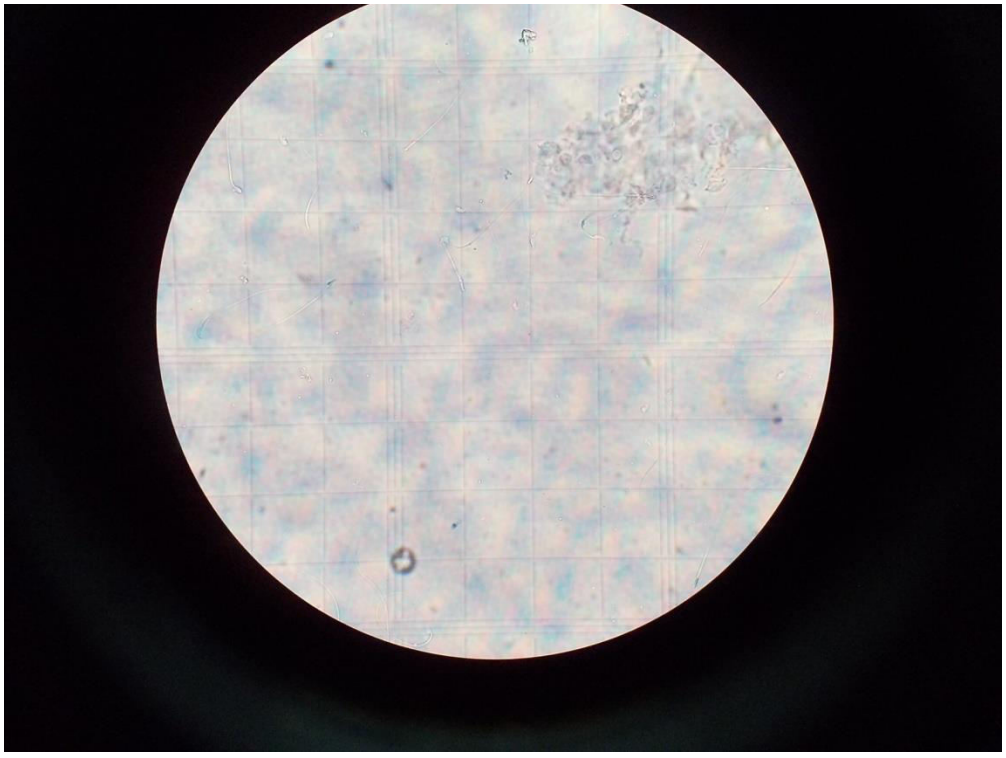

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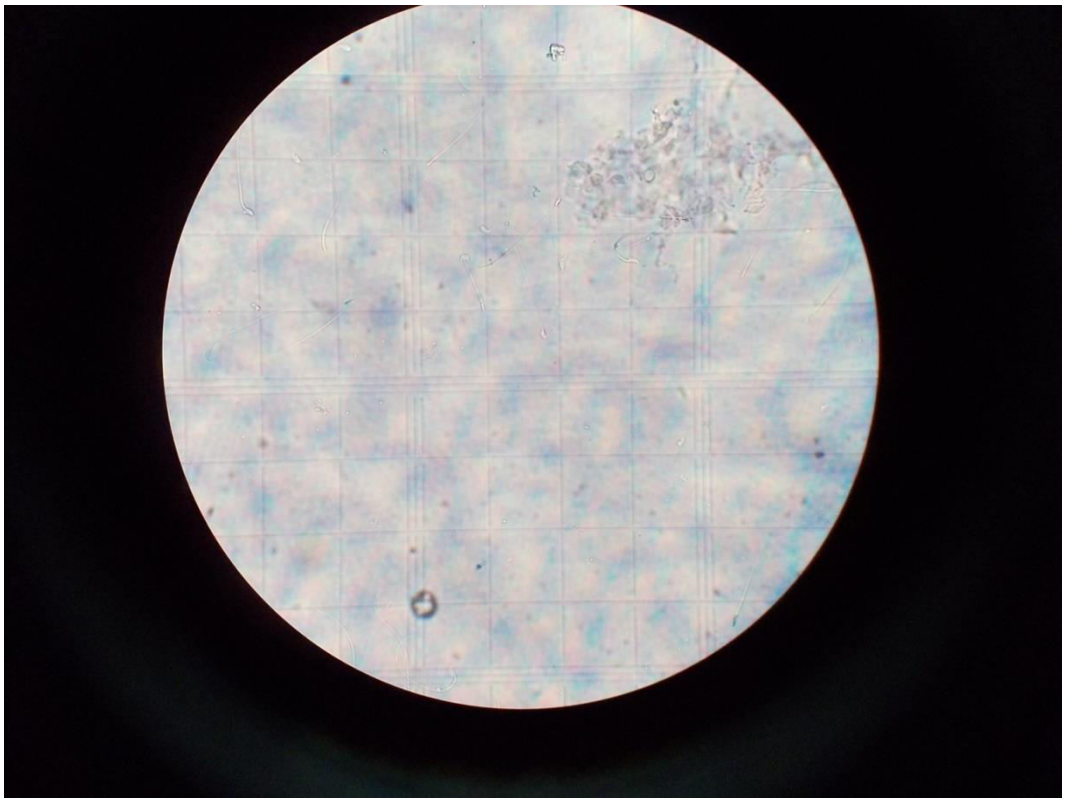

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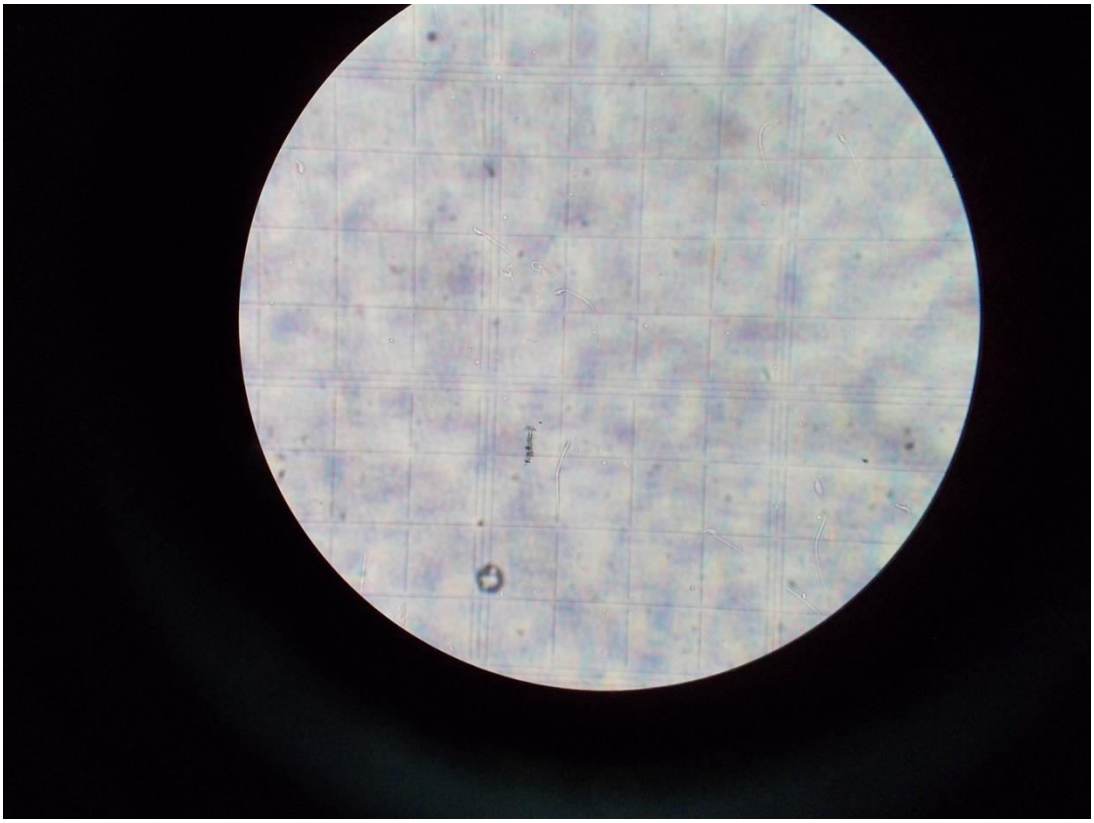

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