

Diagnosis of *Trichomonas vaginalis* Infection and Its Relation to Internal Vaginal Hygiene

Lamia A. Galal¹, Omar M. Shaaban², Ahmed M. Abbas^{2*}, Abo Bakr A. Mitwaly², Andrew N. William² and Tarek K. Al-Hussaini²

¹Department of Parasitology, Faculty of Medicine, Assiut University, Egypt.

²Department of Obstetrics and Gynecology, Faculty of Medicine, Assiut University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. Authors TKA, LAG and OMS designed the study. Author ANW wrote the protocol. Author AMA wrote the first draft of the manuscript. Authors LAG, OMS and AAM managed the literature searches. Author LAG managed the experimental part. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study primarily aims to compare the sensitivity and specificity of OSOM[®] Trichomonas Rapid Test with Giemsa stained direct microscopic examination in the diagnosis of *Trichomonas vaginalis* (TV). The secondary aim is to evaluate the effect of vaginal douching (VD) on the prevalence of TV infection.

Study Design: Cross-sectional observational study.

Place and Duration of the Study: Assiut Women Health Hospital, Egypt between September 2014 and November 2015.

Methodology: All women suspected to have TV were recruited and samples were subjected to Giemsa stained microscopic examination and OSOM[®] Test. A twenty-item questionnaire that included the participants' internal vaginal hygiene performance was completed. The study included 99 participants; fifty of whom performed internal VD.

*Corresponding author: Email: bmr90@hotmail.com;

Results: Of the 99 examined samples, microscopic examination detected 40 positive cases, while OSOM[®] Test detected 57 positive cases ($P = .016$). There was no statistically significant difference between performers and non-performers as regard having positive OSOM[®] Test ($P = .368$).

Conclusion: Internal VD does not affect the incidence of *TV* infection. OSOM[®] Test is superior to direct Giemsa stained microscopic examination in the diagnosis of *TV* infection especially among VD users.

Keywords: *Trichomonas vaginalis*; OSOM[®] *Trichomonas rapid test*; wet mount; vaginal douching.

1. INTRODUCTION

Trichomonas vaginalis (*TV*) is a flagellated protozoon, which is causative agent of trichomoniasis, which is the most prevalent non viral sexually transmitted infection worldwide affecting about 3% of women in USA [1]. Pregnant women with trichomoniasis are at risk of unfavorable birth outcomes such as premature rupture of membranes, premature labor, and low birth weight [2]. Trichomoniasis is associated with a 30% increase in low birth weight infants and a 30% increase in risk of preterm births [3].

Diagnosis of trichomoniasis infection is usually based on microscopic demonstration of the parasites either in wet mount expressing a distinctive jerky movement or by identifying its characteristic morphology in stained smears. Although wet mount is inexpensive and rapid, it is characterized by low sensitivity and mainly depends on examiner's experience or the organism's viability [4].

Currently, the "gold standard" for the *TV* identification is culture. Traditionally, successful *TV* culture requires Diamond's medium, which is not widely available and thus used mainly for research purposes [5]. The OSOM[®] *Trichomonas Rapid Test* (Sekisui Diagnostics, San Diego, CA, USA) is a diagnostic test for *TV* [6].

Vaginal douching (VD) is defined as placing any liquid solution (including water with or without antiseptics) inside the vagina, either by hand, water jet (using a nozzle connected to the water source to introduce water) or a pumping instrument, usually performed for cleanliness after menstruation or intercourse or relief from vaginal itching and irritation [7,8,9]. VD is prevalent practice in Egypt and has traditional and religious roots within the community [10]. Much effort and awareness campaigns are needed to increase women awareness about health hazards of this incorrect practice and to limit its use.

VD increases certain reproductive health hazards especially preterm labor (1.6-1.9 times higher)

[11], ectopic pregnancy (4 times higher) and the risk of pelvic inflammatory disease by 73% [11]. Moreover, VD is associated with recurrent vulvovaginal infections as bacterial vaginosis (1.2-5.1 times higher) [10] and vulvovaginal candidiasis [12], but insufficient studies have evaluated the correlation between VD and *TV* infection.

The aim of the present study was to compare between Giemsa stained microscopic examination and OSOM[®] *Trichomonas Rapid Test* in diagnosis of trichomoniasis in symptomatic women and to evaluate the effect of VD on the prevalence of *TV* infection in reproductive age women.

2. MATERIALS AND METHODS

2.1 Participant Recruitment

A cross sectional observational study was performed from September 2014 to November 2015 in the Department of Obstetrics and Gynecology in collaboration with the Department of Parasitology, Faculty of medicine, Assiut, Egypt.

All consecutive patients who presented to Assiut Women's Health Hospital Outpatient Clinic with clinical impression of *TV* infection were invited to participate in the study. Trichomoniasis was suspected based on patients' presentation with diffuse malodorous yellow-green discharge, dysuria, itching and vulvar irritation [3].

All patients suspected to have *TV* were recruited except those who refused participation in the study. We included reproductive age (18-48 years) patients, with vaginal PH >4.5 and positive Whiff test. However, we excluded patients who had VD in the last 24 hours before sample collection and those under any type of antibiotic therapy in the last two weeks before examination. Pregnant, menstruating women and those with undiagnosed vaginal bleeding were also excluded.

The Ethical Review Board of the Assiut Medical School approved the study protocol. Informed consent was obtained from all study participants.

2.2 Intervention

The study included an Arabic interview administered questionnaire. The final Arabic interview text was validated with eligible participants to determine whether it was acceptable, simple and readily understood. The questionnaire administered by a trained clinic nurse included patients' age, working status, residence, educational level, obstetric history and use of contraceptive method.

All participants were asked about their performance of VD, frequency, methods involved and timing of its performance. Participants were assigned into one of two groups: according to whether or not they performed VD. After group assignment, all participants underwent vaginal swab collection for Giemsa staining and OSOM[®] Trichomonas Rapid Test.

2.2.1 Samples collection

Standard Precautions were followed when collecting and handling potentially infectious specimens. The vaginal secretion was collected using a sterile Cusco's speculum and sterile vaginal swab, with long handle to easily reach the posterior fornix without touching the vagina, by rubbing the vaginal wall or collecting material from the posterior fornix. Three vaginal swabs were collected; the first used for determination of vaginal PH, Whiff test. Only those with vaginal PH > 4.5 and positive Whiff test, the two other swabs were processed for microscopic examination and OSOM[®] Trichomonas Rapid Test. The sample was maintained at room temperature and analyzed within 15 minutes.

2.2.2 PH determination

Using a circular motion, the vaginal swab was gently applied over the surface of the PH test paper (VWR International, West Chester, PA, USA) and immediately the color reaction on the paper was observed and compared to the color comparison chart provided by the manufacturer to determine the PH of the sample. Then the results were recorded on the sheet [13].

2.2.3 Whiff test for amines

A portion of vaginal swab was applied to the surface of a clean glass slide then one drop of

10% KOH was added directly to the vaginal sample. The slide was held gently to fan the vapor layer (Whiff) above its surface and assessed for the presence of volatile amines which have a fishy odor. The results were recorded on the sheet as positive if there was a fishy odor following addition of KOH to the vaginal sample and negative if there is no fishy odor.

2.2.4 Microscopic examination

The vaginal swabs were smeared on clean dry microscopic slides, allowed to dry and fixed with methanol then stained with Giemsa stain. The slides were observed under 400X power for TV characteristic morphology. TV does not always appear in its typical pear-shaped forms but may resemble polymorph nuclear leucocytes.

2.2.5 OSOM[®] trichomonas rapid test

OSOM[®] Trichomonas Rapid Test is an innovative CLIA-waived, point of care diagnostic manufactured by Sekisui Diagnostics, San Diego, CA, USA. This test uses a color immunochromatographic, capillary flow, "dipstick" technology and detects parasite antigens directly from vaginal swabs. No microscope is needed. Results are available within 10 minutes of administration.

The test was performed according to the manufacturer instructions; the third collected secretion was placed in the kit's sample buffer test tube to solubilize Trichomonas proteins that may be present in the sample. The tube was labeled with a patient identifier for OSOM[®] Trichomonas Rapid Test. The dipstick was then placed in the sample mixture. If TV antigens were detected, they react with anti-Trichomonas antibody in the stick. A visible blue test line along with red control line indicated a positive result.

2.3 Data Collection and Analysis

The collected data were entered on Microsoft access data base to be analyzed using the Statistical Package for Social Science (SPSS Inc., Chicago, version 21). Comparisons between the groups were done using Student's t-test to compare the mean values between groups in scale variables. However, χ^2 test was used to compare the dichotomous and ordinal variables in the groups. For analysis P<0.05 was considered significant.

3. RESULTS AND DISCUSSION

One-hundred thirty two patients diagnosed clinically to have *T. vaginalis* infection with vaginal PH > 4.5 and Positive Whiff test were approached to participate in this study. Thirty-three women were excluded: eight women were pregnant, eleven were performed VD in the last 24 hours prior to evaluation, and six had received antibiotic treatments in the prior 2 weeks. Moreover, eight women refused to participate in the study. The remaining 99 women were included in the study; fifty of them were performing VD while 49 were non-performers (Fig. 1).

Internal VD was performed by 50.5% of the studied women who had trichomoniasis. The mean age of patients was 32.19±6.37 years. Most of them were housewives (91.9% of total cases). There were no significant differences between women who had performing VD or not as regard working status, residence, level of education and number of previous deliveries, abortion, living children. With regard to contraceptive use, 51.5% of participants were using contraceptive method at the time of the

study with non-significant difference between users and non-users of VD (Table 1).

With regard to confirmatory diagnosis of TV, 57 cases (57.6%) were positive by OSOM[®] Trichomonas Rapid Test while Giemsa stained microscopic examination diagnosed only 40 cases (40.4%). A clinically and statistically significant difference ($P=.016$) was present. When comparing the accuracy of microscopic examination versus OSOM[®] Trichomonas Rapid Test, we found that the sensitivity, specificity, positive predictive negative predictive values of Giemsa stained microscopic examination were 59.6, 85.7, 85.0 and 61.0 % respectively.

Table 2 comparing the percentage of positive cases either by OSOM[®] Trichomonas Rapid Test or Giemsa stained microscopic examination and the history of VD. Our results shows that 62% of participants using VD were OSOM[®] Trichomonas Rapid Test positive but only 46% of were positive for TV by microscopic examination with no statistically significant between VD users and non-users as regard incidence of having positive tests ($P=.368, .252$) for OSOM[®] Trichomonas Rapid Test and microscopic examination.

Table 1. Demographic characteristics and contraceptive history of women performing vaginal douching or not

Total cases	Vaginal douching			P-value
	Yes 50 (50.5%)	No 49 (49.5%)	Total 99	
Age (years) (mean±SD)	33.18±6.02	31.18±6.52	32.19±6.37	0.45
Working status				0.479
Not working	45 (90)	46 (93.9)	91 (91.9)	
Working	5 (10)	3 (6.1)	8 (8.1)	
Residence				0.846
Rural	39 (78)	39 (79.6)	78 (78.8)	
Urban	11 (22)	10 (20.4)	21 (21.2)	
Educational level				0.201
Illiterate	20 (40)	23 (46.9)	43 (43.4)	
Primary	7 (14)	7 (14.3)	14 (14.2)	
Secondary	9 (18)	14 (28.6)	23 (23.2)	
University	14 (28)	5 (10.2)	19 (19.2)	
Parity (mean±SD)	3.1±2.22	3±2.4	3±2.3	0.875
Abortion (mean±SD)	1.07±0.7	1.52±0.98	1.32±0.85	0.062
Number of children (mean±SD)	3.04±2.12	2.88±2.11	2.96±2.1	0.906
Type of contraceptive method				
Non use	25 (50)	23 (46.9)	48 (48.5)	0.336
IUD	14 (28)	8 (16.3)	22 (22.2)	0.062
Oral contraceptives	7 (14)	3 (6.1)	10 (10.1)	0.053
Injectables	2 (4)	8 (16.3)	10 (10.1)	0.06
Implants	1 (2)	4 (8.3)	5 (5)	0.082
Condoms	1 (2)	3 (6.1)	4 (4)	0.21

Douching: mean woman who do perform internal vaginal douching by fingers or pumps. **No douching:** women who were not perform any internal vaginal hygiene. **IUD:** Intrauterine device.

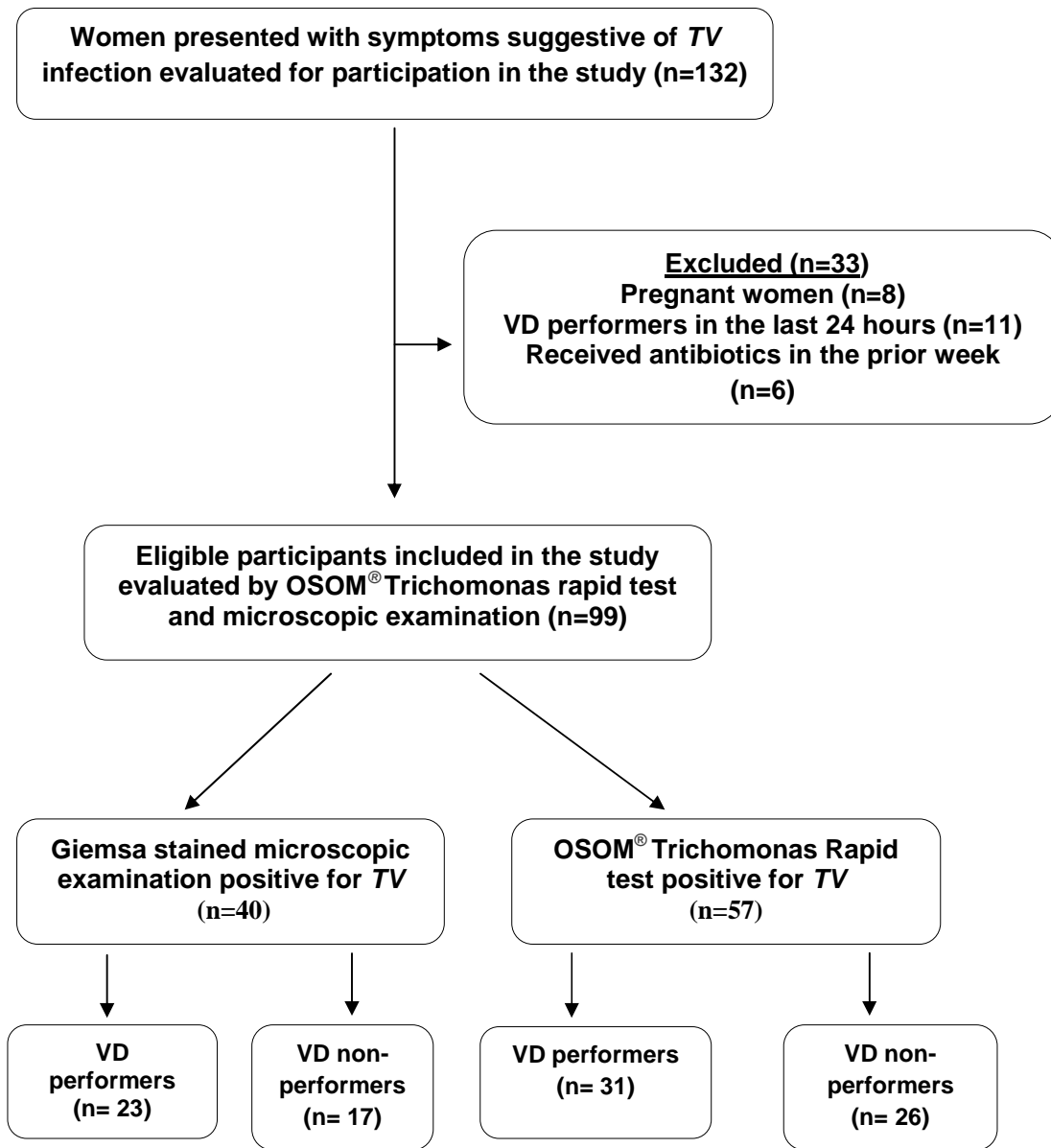


Fig. 1. The study flowchart

Table 2. Relation between vaginal douching and TV infection detected by OSOM® Trichomonas test and Giemsa stained microscopic examination

	OSOM® Trichomonas test		P-value	Microscopic examination				P-value	Inter-test comparison P-value		
	Positive			Positive		Negative					
	No.	%		No.	%	No.	%				
Vaginal douching											
Yes	31	62.0	19	38.0	0.368	23	46.0	27	54.0	0.252	0.016*
No	26	53.1	23	46.9		17	34.7	32	65.3		
Total	57	57.6	42	42.4		40	40.4	59	59.6		

* Significant value ($p < 0.05$)

Table 3. Relation between frequency and timing of vaginal douching and the results of OSOM[®] Trichomonas test

	OSOM [®] Trichomonas test				P-value
	Positive		Negative		
	No.	%	No.	%	
Vaginal douching	31	62.0	19	38.0	0.368
Frequency of vaginal douching					
Once per day	16	51.6	8	42.1	0.514
More than once	15	48.4	11	57.9	0.608
Timing of vaginal douching					
After urination or defecation	8	25.8	5	26.3	0.968
After intercourse	1	3.2	1	5.3	0.721
Before pray	6	19.4	5	26.3	0.822
No fixed time	16	51.6	8	42.1	0.514

Table 3 show that there are no significant differences among VD users in the results of OSOM[®] Trichomonas Rapid Test as regard frequency or timing of VD.

The current study demonstrated that the OSOM[®] Trichomonas Rapid Test is more sensitive over the microscopic examination in diagnosis of trichomoniasis. Internal VD was performed by about 50.5% of the patients that presented to the outpatient clinic in Assiut, Egypt with trichomoniasis, but there is no effect of VD on the presence of trichomoniasis.

Trichomoniasis is the most prevalent sexual transmitted infection [1]. Globally, the World Health Organization (WHO) estimates that 276 million new cases of trichomoniasis are diagnosed annually [14]. Little emphasis has been made on the importance of decreasing the rates of trichomoniasis despite ease of treatment [1]. Emergence of trichomoniasis as a cofactor for HIV transmission (increase HIV acquisition by RR, 2.57; 95% CI, 1.42–4.65) [15] and vertical transmission to newborns (rate 2-17%) [16].

Currently, wet mount microscopic examination and syndromic diagnosis based on the criteria of the vaginal discharge are the most widely used methods for diagnosing and treating TV in resource-constrained settings. However; wet mount microscopic examination requires equipment and trained technicians. The OSOM[®] Trichomonas Rapid Test is a simple and objective diagnostic assay expected to improve the diagnosis of trichomoniasis, especially in settings where microscopy and culture are unavailable [6].

The diagnosis of trichomoniasis in women is often made by wet mount smear of a vaginal fluid specimen or by an incidental finding on a Papanicolaou test report. Despite the fact that there is high specificity reached up to 98.0% to 100.0% for these tests, their sensitivity may be low ranging from 38.5% to 60.0% [17,18]. This was the rationale behind using Giemsa stained microscopic examination instead of wet mount smear for initial diagnosis of trichomoniasis in our study.

The current study included 99 women with clinical manifestations of trichomoniasis. Giemsa stained microscopic examination was positive in about 40% of the clinically suspected cases with PH > 4.5 and positive for Whiff test. In previous studies, the percentages of women diagnosed positive during direct wet mount microscopic examination were lower than that in our study. It was 15.7% in the study of Huppert et al. [6] in USA, 18 % in the study of Patil et al. [17] in Belgium and 32.9% in the study of Hegazy et al. [19] in Egypt.

The difference may be due to the nature of the included participants. In our study we limited testing by microscopic examination to those with high PH >4.5 and positive for Whiff test, while in the other studies they had screened all women with abnormal vaginal discharge. In the present study we could not calculate accuracy test of microscopic examination, as the gold standards of culture or PCR had not been done.

Other diagnostic option includes OSOM[®] Trichomonas Rapid Test, which has moderately higher sensitivity. However, this option not used routinely and not cost-effective for many clinicians [17]. In a study done by Zaki et al [17]

in Egypt, the sensitivity and specificity of OSOM[®] Trichomonas Rapid Test were 85.7%, 99.1% respectively. In another study for evaluating OSOM[®] Trichomonas Rapid Test, the test showed sensitivity of 84%, specificity of 98% [20], 83.3 and 98.8%, respectively [6].

In the present work OSOM[®] Trichomonas Rapid Test were positive in about 57.6% of clinically suspected cases with PH>4.5 and positive Whiff test. In previous studies, the percentage of women diagnosed positive by OSOM[®] Trichomonas Rapid Test was about 43.78% in the study of Hegazy et al. [18]. On the other hand, much lower percentage had been obtained from women with abnormal vaginal discharge, reported by Zaki and colleagues [18]. This study showed that 10.16% of their studied participants were positive using the test.

These results were much lower than our results secondary to limiting doing OSOM[®] Trichomonas Rapid Test in our study to those with positive whiff test and had PH >4.5. In a recent study [21] in which OSOM[®] Trichomonas Rapid Test were done as a screening test of the general population in India, 13.8% women (62/450) were diagnosed with trichomoniasis.

In the present work we compared the diagnostic accuracy of Giemsa stained microscopic examination against OSOM[®] Trichomonas Rapid Test, the sensitivity of microscopic examination was 59.6% and specificity was 85.7%. VD may washes the parasite from the vagina but not wash the antigen, makes antigen detection more accurate. This is the explanation to how OSOM[®] Trichomonas Rapid Test detected more cases of TV than microscopic examination.

Vaginal douching is a common practice among women all over the world and is used for personal hygiene or other religious reasons in many countries [7]. Half of our participants were performing internal VD and the rest were not. VD is a prevalent practice in Egypt; some women consider it part of their daily practice of for purifying before prayers. Previous studies in Assiut, Egypt showed 73% of married females were performing VD [10]. A reproductive hygiene program that was done in our locality after the previous results in the same setting may be behind the lower rate of performance of this habit. However, in other setting they reported about 43% of African-American adolescence in USA performing VD [22]. On the other hand, about

three quarters of married women in Nairobi, Kenya perform VD [23].

Our results showed that 62% of VD performers tested positive with OSOM[®] Trichomonas Test, while microscopic examination tested positive in only 46% of TV infections. These figures in spite of showing some higher incidence of TV in VD performers, the difference did not reach a significant value. Increasing sample size may allow getting some different significant difference.

There were three previous studies examined the relation between VD and TV infection. Two of them were in accord to our results of no association between TV and VD. One in Kenya found that VD was not significantly different in women infected by TV 64/381 (17.0%) in VD performers versus 22/147 (15.0%) in non-performers [23]. The other study in USA found that women infected by TV were 1.2% in performers versus 0.8% [24].

The third study in USA [24] found an association between VD, TV and HIV higher among those who douched about once a week compared to those who did not douched. This study had a number of limitations; its cross-sectional nature which can neither refute nor confirm whether higher rates of STIs in African-American youth were associated with douching causally or because of STI symptoms result in more douching [24]. More studies are needed to confirm this association. However, there is a big difference in the hygienic habits of women between the two cultures, as the prevalence of STI is quite low in Egypt.

The current study had faced some limitations; first, cross-sectional design. Second, inability to perform the gold stranded test of culture or PCR. Third, the limited number of recruited cases due to limitation in the availability of the test. Fourth, lack of external validity as our results are not generalizable or comparable to other populations as we included only women who presented to the outpatient gynecology clinic. Fifth, we did not evaluate the cost-effectiveness of using the OSOM[®] Trichomonas Rapid Test in our study.

Further research is needed with good power to compare the sensitivity of OSOM[®] Trichomonas Rapid Test if done with or without preliminary whiff test and PH evaluation. Moreover, cost analysis need to be addressed. Further studies with bigger sample size to evaluate the relation

of the TV with all reproductive health hazards are also needed.

4. CONCLUSION

In conclusion, VD does not affect the frequency of trichomoniasis. OSOM[®] Trichomonas Rapid Test is superior to Giemsa stained microscopic examination in diagnosis of trichomoniasis especially among VD users. OSOM[®] Trichomonas Rapid Test is simple bed side requires minimal training with relatively good sensitivity but expensive. Limiting testing to those cases with positive whiff test and with PH >4.5 may much reduce these costs.

CONSENT

All authors declare that 'written informed consent was obtained from all patients.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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