

Evaluation of the sensitivity of Faust method and spontaneous sedimentation for the diagnosis of giardiasis

Evaluación de la sensibilidad de los métodos Faust y sedimentación espontánea para el diagnóstico de giardiasis

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ABSTRACT

Introduction: in a routine clinical laboratory, parasitological methods are used for the detection and diagnosis of intestinal parasitic diseases.

Objective: To evaluate the sensitivity of Faust's and the spontaneous sedimentation methods (Hoffman, Pons and Janer).

Methods: a comparative study was developed. The analyzes were made on control samples containing *Giardia lamblia* from the Laboratory of Clinical Pathology of Santa Casa de Misericórdia of São Paulo and processed again in the clinical laboratory at the University of Nove de Julho by 18 analysts.

Results: the statistical difference estimated by the McNemmar test ($p= 0.0156$) and the Bland and Altman statistics through the graphical analysis showed low correlation between the techniques applied in this research, which indicates that the Faust technique is the most sensitive for the detection of protozoan cysts.

Conclusions: this paper is relevant for the quality control of laboratory procedures to be applied to minimize the risk of false positives and false negatives.

Keywords: *Giardia lamblia*; spontaneous sedimentation; centrifugal-flotation; Bland-Altman Plots.

RESUMEN

Introducción: en un laboratorio clínico de rutina, los métodos parasitológicos se utilizan para la detección y diagnóstico de las enfermedades parasitarias intestinales.

Objetivo: evaluar la sensibilidad de los métodos Faust y sedimentación espontánea (Hoffman, Pons y Janer).

Métodos: Un estudio comparativo fue diseñado. Los análisis se realizaron con muestras de control que contienen *Giardia lamblia*, procedentes del Laboratorio de Patología Clínica de la Santa Casa de Misericordia de Sao Paulo y procesadas de nuevo en el Laboratorio Clínico en la Universidad Nueve de Julio por 18 analistas.

Resultados: la diferencia estadística mediante la prueba McNemmar ($p = 0,0156$) y las estadísticas de Bland y Altman a través del análisis gráfico mostraron bajas correlaciones entre las técnicas utilizadas en este estudio, lo que sugiere que la técnica de Faust es más sensible para la detección de quistes de protozoarios.

Conclusiones: este trabajo es relevante para el control de calidad de los procedimientos de laboratorio que deben aplicarse para reducir al mínimo el riesgo de falsos positivos y falsos negativos.

Palabras clave: *Giardia lamblia*; sedimentación espontánea; centrífugo-flotación; Bland y Altman.

INTRODUCTION

Intestinal parasites are considered a serious public health problem due to its high prevalence in poor countries.¹⁻⁶ Most intestinal parasites are detected and identified by fecal exams, which are quantitative and or qualitative methods, able to identify the different stages of parasites morphology.⁷

The diagnostic of intestinal parasites have, on this tests, a feasible method, which is also used as the gold standard for many diseases e.g. the final diagnosis and cure control of schistosomiasis.^{8,9} According *Gargioni et al.*, 2008¹⁰ parasitological techniques have disadvantages because the sensitivity varies considerably, depending on the amount of examined faeces, the number of eggs or cysts excreted, intrinsic factors inherent in the loss during the procedure and accuracy of the laboratory technician. Thus, the difficulty of diagnosis by a single method reduces the diagnostic sensitivity.¹⁰

In a reference analysis on the diagnosis techniques of intestinal parasitosis, we observed on the work of *Tibiriça et al.*¹¹ a divergence between the techniques and their application on parasitic infections diagnosis, not corroborating with the text of *Uecker et al.*,¹² which states that, for the diagnosis of parasitic diseases, the stool test is considered fundamental, although often not given due attention by health professionals who, in most cases, are dedicated to more sophisticated and expensive diagnostic techniques or even dispense the parasitological exams in favor of prophylactic or presumptive treatment.¹²

Among the techniques that are used, the fecal exam performed by the sedimentation method developed by Hoffman, Pons and Janer (HPJ),¹³ also known as the Lutz technique is cited in most of scientific papers, alone or associated with other parasitological methods.¹⁴ It is interesting to note that the researchers (HPJ), had as main concern the intestinal schistosomiasis diagnosis in Puerto Rico and the discussion was focused on the amount of faeces to be analyzed, consistency and sedimentation optimization.¹³

Another wide application technique in the studies surveyed, easy to perform and low cost, was developed by Faust,¹⁵ which applies centrifugal-flotation methods with a 33 % zinc sulfate solution being considered the choice method for diagnosis of lightweight structures of intestinal parasites such as protozoan cysts including veterinary relevance.¹⁶

Often, the laboratory routine and standard operational procedures are not appropriate to the epidemiological profile of the population, the techniques may not have the best diagnostic sensitivity with different parasites and false negative results may occur frequently.

Accordingly, we statically evaluated the efficacy of Faust and HPJ techniques for the diagnosis of giardiasis and the diagnostic sensitivity of laboratory workers when using these methods. The techniques were chosen because they showed positive results in previous studies,^{1-4,17} as well as being easy techniques to perform, routine use and low cost. The protozoan *Giardia lamblia* was the element to be searched because it is the most common protist parasite in epidemiological surveys, with morphological features easily detectable in fecal samples and also because there is no reference to applicability of the HPJ technique as a sensitive method against this parasites.

METHODS

The study was performed at the clinical laboratory of the University Nove de Julho (UNINOVE), São Paulo, with 17 analysts from 7th and 8th periods, studying Biomedicine, Biology and Pharmacy, at the 2nd half of 2011.

The positive samples (by methods Faust and HPJ) were provided by the Clinical Pathology Laboratory of the Santa Casa de São Paulo, Parasitology sector, which had no indication of its origin, being identified by numbers (1-6).

Each analyst removed the same amount of samples (about 10 grams per sample) and conducted the manipulation techniques as indicated by De Carli.⁷ Readings were done in triplicate for 3 minutes between each slide by technique and by reader; 204 examinations were performed (6 samples x 17 analysts x 2 techniques)

with a total of 612 slides analyzed. The counting of cystic structures was done by reading field sweep in 3 min of observation.

Statistical analyzis were performed using the McNemmar adjustment test¹⁸ which the paired data were evaluated and organized regarding the concordance. Were classified as zero (number of cysts < than 35) and 1 (number of cysts equal or > than 35) in order to detect significant statistical differences ($\alpha = 0.05$), with a 95 % confidence interval.

This test was applied due to the non-parametric profile observed by the Kolmogorov-Smirnov test, where the relative frequency was compared as % detection of Giardia cysts, using as a model before and after the analyzes done by Faust and HPJ techniques and the quantity of observed cysts.

To observe the difference between the mean sensitivity along the diagnoses, we applied the method developed by Bland-Altman¹⁹ where the confidence limits are specified as bias + 1.96 STD (mean difference + 1.96 x standard deviation of the difference). In this technique, the X axis in the graph is the average of the values, and the Y axis reports the mean and standard deviation of the differences.

In all statistical tests described, we used the statistical software business Medcalc ® Version 12.1 for data analysis.

RESULTS

The number of cysts found on Faust method was relatively larger than the number of *G. lamblia* cysts found on HPJ method (855 and 308 respectively). Among the statistical evaluations, a significant difference was observed between the methods used and the accuracy of laboratory workers. The results can be seen in [table](#), where the McNemmar test returned a value of $p = 0.0156$, being the factor before, designated as the number of cysts observed in the Faust technique and the factor after the analysis of the same samples by the HPJ technique.

In the Bland-Altman chart ([Fig.](#)), no correlation was observed between the analysts according to the mean number of cysts detected on microscopy.

Table. McNemar test result between the sensitivity of HPJ and Faust techniques

	Method Faust	Method de HPJ	IC (95 %)
Classification 0	7	0	7.44-41.18
Classification 1	7	3	
Total	14	3	

$p = 0.0156$.

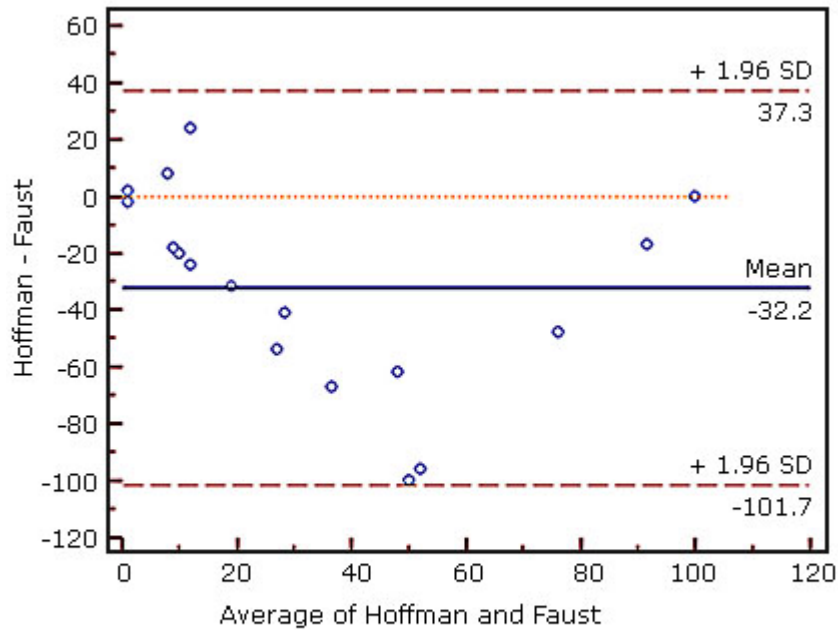


Fig. Agreement between Laboratories in the diagnosis of *G. lamblia* cysts by HPJ and Faust techniques evaluated by Bland and Altman graphical modeling.

DISCUSSION

The centrifugal-flotation technique at 33 % zinc sulfate was more sensitive than the spontaneous sedimentation, as shown by other studies about the diagnosis of giardiasis in fecal samples. The results observed in the McNemar statistical calculations showed a better application of the Faust technique when repeated the same samples by the HPJ technique.

All samples were positive, with a high concentration of cysts, and the laboratory conditions, in which the experiment was conducted, were identical to those involved in the analysis, as well as the organoleptic characteristics of the stool (solid).

The routine of a clinical laboratory of parasitology must commune an arsenal of techniques according to the endemicity of most parasites observed in the population served. At the results obtained, we found that the Faust technique showed better sensitivity and significant statistical differences between the number of cysts detected, as seen in table.

Giardiasis is a endemic parasitic disease in both urban and rural areas, and presents asymptomatic forms in adults and a varied transmission cycle which can show a huge relevance subclinical universe.^{1,5,20,21}

The difference between the number of cysts observed by analyst states shows that detection can change among laboratory workers, even using the same samples.

Schistosomiasis and other parasitic diseases such as oxyuriasis should also be searched by specific and quantitative methods, which can thus have a vision of health emergency or reemergence of these important diseases that primarily show negligence health and social, which still lives good portion of the population.

This way we must remember that the quality control and technical training should be a routine inside the laboratory. More sensitive and specific new techniques must be researched within universities and among the public or private diagnostic services.

Only one method does not provide good reliability, but not always the laboratories count the samples number in appropriate conditions, remembering that the life cycles of parasites within the human host also involves its oviposition periodicity or formation of new cysts.

Random medication may mask the data and leading to an increase of false negative results, making it more difficult the application of epidemiological studies that can make a profile of intestinal parasites in both rural and urban areas.

The results of this research show that there is a large difference in diagnostic sensitivity by both the technique and the analyzer

The results of this research show that there is a great difference in the diagnostic sensitivity both by the technique and by the analyzer. Minimizing possible errors, developing new methods for the diagnosis of intestinal parasites that increase sensitivity and specificity is a constant concern for ensuring a good diagnostic model and better results for epidemiological surveys regarding these permanent public health problems.

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