

## A SIMPLE DEVICE FOR QUANTITATIVE STOOL THICK-SMEAR TECHNIQUE IN SCHISTOSOMIASIS MANSONI

Naftale KATZ<sup>(1)</sup>, Adelú CHAVES<sup>(1)</sup> and J. PELLEGRINO<sup>(2)</sup>

### SUMMARY

A disposable piece of 1.37 mm-thick cardboard with a hole (6 mm in diameter) in the center was used to have fecal samples in regular amounts for quantitative determination of eggs of *Schistosoma mansoni* by the Kato's method. Each of 10 different human stool samples were used to prepare 10 slides. The results showed that the mean weight of the feces was  $43.7 \pm 2.8$  mg with a coefficient of variation of 6.4%. Feces from 100 patients with schistosomiasis mansoni were used to prepare 2 slides for each fecal sample: one by the cardboard technique and the other by weighing a sample of 45 mg. The coefficient of correlation between the number of schistosome eggs per gram of feces obtained by the cardboard technique and the conventional weighing procedure was very high ( $r = 0.813$ ;  $p > 0.01$ ). The method described was considered to be quite useful for mass surveys on schistosomiasis, due to its reliability, low cost and easy performance.

### INTRODUCTION

A cellophane thick-smear technique introduced by KATO & MIURA<sup>3</sup> and further improved and evaluated by KATO<sup>2</sup>, KOMIYA & KOBAYASHI<sup>5</sup>, MARTIN & BEAVER<sup>7</sup>, CHAIA et al.<sup>1</sup>, and KATZ et al.<sup>4</sup> is now widely used. According to these Authors the thick-smear technique is very simple and highly effective for detecting helminth eggs in the feces, especially of schistosomes. However, when the aim is to determine quantitatively the number of eggs it is necessary to weigh the fecal samples to be examined. This is time consuming and requires a precision balance, which makes the procedure rather difficult to be carried out under field conditions.

Recently, LAYRISSE et al.<sup>6</sup> suggested the use of a 1-ml calibrated plastic syringe which is sectioned 3 mm from its tip to ensure a constant displacement of the plunger and hence

ing a constant volume of stool. Such device makes it possible to estimate the volume of feces brought for examination by Kato's technique, and is claimed to be "useful in public-health programs, due to its low cost and the re-usability of the syringe". In our opinion, however, the re-usability of the syringe constitutes a great disadvantage. The necessity of washing the testing material is time consuming and also could be responsible for contamination of fecal samples by helminth eggs.

This paper presents the data provided by a simple device for obtaining without scales uniform weights of fecal samples.

### MATERIAL AND METHODS

A fecal measuring device was constructed of rectangular cardboard (3 cm x 4 cm x

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- (1) "Centro de Pesquisas René Rachou", "Instituto de Endemias Rurais" and "Seção de Parasitoses da Prefeitura", Belo Horizonte, Brazil
- (2) "Centro de Pesquisas René Rachou", and "Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais"

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1.37 mm) by making a hole 6 mm in diameter in its center. Feces, previously strained through 105 mesh stainless-steel cloth (W. S. Tyler Co., Cleveland, Ohio), are placed in the hole by tooth-pick transfer. When the feces are leveled and the device removed from the microscope slide beneath, the measured sample remains.

Each of 10 different human stool samples were used to prepare 10 slides. The slides were weighed in an analytical balance (1 mg sensitivity) before and after the stool samples were added using the cardboard as above mentioned. This was done in order to know the range of variation of the weight of fecal material that filled the hole in the cardboard.

For further evaluation, feces from 100 patients with schistosomiasis mansoni were used to prepare 2 slides for each fecal sample: one by the cardboard technique and the other by weighing a sample of 45 mg. After the cardboard was removed from the slide, or the feces weighed, a cellophane coverslip, previously soaked in Glycerine-malachite-green solution (according to MARTIN & BEAVER<sup>7</sup>) was put over the fecal sample. The slide was then inverted and pressed down, so that the smear could spread over an area of 20-25 mm in diameter. The preparations were kept at room temperature for 1-2 hours and then examined under the microscope. After counting the eggs in each slide, the number of eggs per gram of feces was obtained by multiplying the figure by 1,000 and dividing by the fecal weight.

For the statistical evaluation of the data the coefficient of variation and the standard deviation were calculated. The coefficient of correlation between the figures provided by the cardboard technique and by weighing the fecal samples was also determined.

## RESULTS

As can be seen from Table I, the mean weight of the feces was 43.7 mg (ranging from 42.5 to 45.4), with a mean standard deviation of 2.8 (1.9 to 5.2) and a coefficient of variation of 6.4% (4.5 to 7.4). The analysis of variance showed no statistical significant difference, at 95% level of probability, between the data obtained with the different stool samples.

TABLE I

Reproducibility of weight of stool samples using the perforated cardboard technique

Stool number	Mean weight of fecal samples (mg) and standard deviation (*)	Coefficient of variation
1	43.7 ± 2.8	6.4
2	44.3 ± 2.3	5.2
3	42.5 ± 1.9	4.5
4	42.7 ± 2.7	6.3
5	43.2 ± 3.2	7.4
6	43.5 ± 2.9	6.7
7	43.9 ± 2.9	6.6
8	45.4 ± 3.2	7.0
9	45.0 ± 2.3	5.1
10	43.4 ± 2.9	6.7
Total	43.7 ± 2.8	6.4

(\*) Each figure represents the mean of 10 samples

The coefficient of correlation between the number of schistosome eggs per gram of feces obtained by the cardboard technique and the conventional weighing procedure was very high ( $r = 0.813$ ;  $p > 0.01$ ; Fig. 1) indicating that the former method can be used with great confidence. The total numbers of schistosome eggs per gram of feces found in the 100 patients were 25,146 and 24,815 by the weighing procedure and by the cardboard technique, respectively.

## DISCUSSION

The weights of fecal samples obtained by the cardboard technique were found to be acceptably uniform statistically. The mass of feces obtained is similar to that recommended by previous Authors<sup>4, 6</sup>.

Considering that 43.7 mg represent the weight of the fecal sample using the perforated cardboard, the number of schistosome

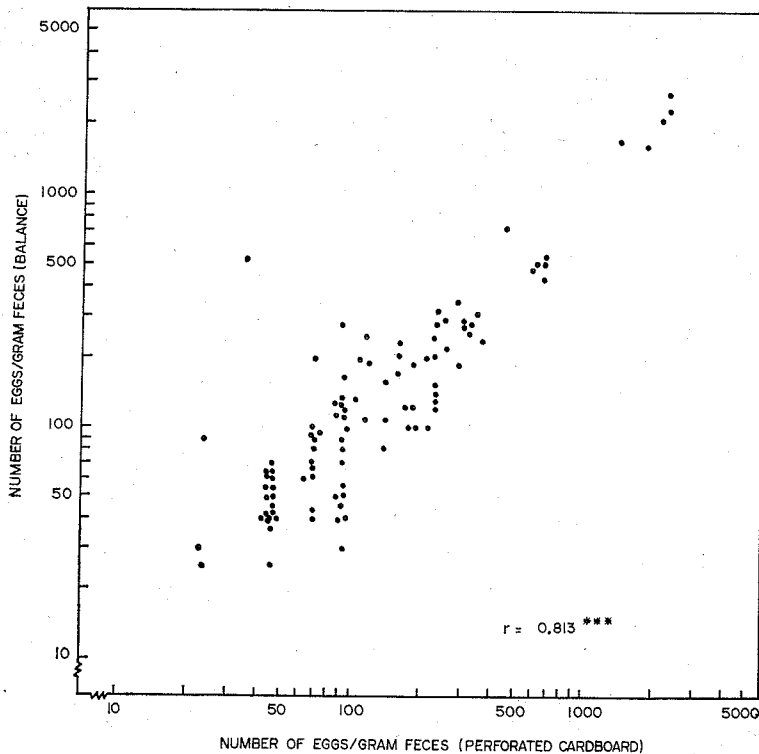


Fig. 1 — Diagram of dispersion from the data obtained with the conventional technique (balance) and the cardboard device.

(\*\*\*) Coefficient of correlation;  $p > 0.01$

eggs per gram can be easily obtained by multiplying by 23 the number of eggs counted per slide.

According to SCOTT & HEADLEE<sup>8</sup> the correction of helminths counts figures for the consistency of stools as recommended by STOLL & HAUSHEER<sup>9</sup> is unnecessary.

The method here described will be quite useful for mass surveys on schistosomiasis, due to its reliability, low cost and simple performance.

#### RESUMO

*Uma técnica simples para a realização do exame de fezes quantitativo na esquistossomose mansônica*

Foi descrita uma nova técnica que permite avaliar o peso das fezes a serem examinadas pelo método de Kato quantitativo, sem que seja necessário o uso de balança.

Foi utilizado um papelão (1,37 mm de espessura) em forma retangular (4 x 3 cm) tendo um orifício de 6 mm de diâmetro no centro. Colocando o cartão sobre uma lâmina de microscopia enche-se o orifício com fezes por meio de um palito de madeira, após tê-las passado por uma peneira de 105 malhas por polegada quadrada. O cartão era então retirado e despezado.

Foram estudadas 10 amostras de fezes, sendo que de cada amostra eram feitas 10 lâminas utilizando-se o cartão perfurado. O peso das fezes contido nestas lâminas foi calculado pela diferença entre o peso de cada lâmina com o material e sem o mesmo. A média dos pesos das fezes nas 100 preparações foi de 43,7 mg, variando de 42,5 a 45,4 mg, com um coeficiente de variação de 6,4%.

Em 100 pacientes que eliminavam ovos de *S. mansoni* foi realizado o método de Kato quantitativo tendo sido preparadas 2 lâminas de cada amostra de fezes. Numa das

lâminas foram colocados 45 mg de fezes, pesados em balança analítica. Na outra lâmina a amostra fecal foi preparada pela técnica do cartão perfurado, considerando-se o peso das fezes como sendo de 43,7 mg. Em seguida as fezes foram recobertas com laminula de celofane, previamente embebida em solução de glicerina-verde de malaquita, e examinadas ao microscópio 1 a 2 horas depois de preparadas. Os resultados obtidos mostraram um coeficiente de correlação elevado,  $r = 0,813$  ( $p > 0,01$ ), indicando que as técnicas do cartão perfurado e da pesagem em balança são equivalentes.

A nova técnica descrita, devido à sua sensibilidade, baixo custo e facilidade de execução poderá ser utilizada, com grandes vantagens, na realização do exame de fezes quantitativo pelo método de Kato, em programas de Saúde Pública.

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#### REFERENCES

1. CHAIA, G.; CHAIA, ANY B. Q.; MCAULLIFE, J.; KATZ, N. & GASPER, D. — Coprological diagnosis of schistosomiasis. II — Comparative study of quantitative methods. *Rev. Inst. Med. trop. São Paulo* 10:349-353, 1968.
2. KATO, K. — A correct application of the thick-smear technic with cellophane paper cover. *Pamphlet*, 9 pp., 1960.
3. KATO, K. & MIURA, M. — Comparative examinations. *Jap. J. Parasit.* 3:35, 1954.
4. KATZ, N.; COELHO, P. M. Z. & PELLEGRINO, J. — Evaluation of Kato's quantitative method through the recovery of *S. mansoni* eggs added to human feces. *J. Parasit.* 56: 1032-1033, 1970.
5. KOMIYA, Y. & KOBAYASHI, A. — Evaluation of Kato's thick-smear technic with a cellophane cover for helminth eggs in feces. *Jap. J. Parasit.* 19:59-64, 1966.
6. LAYRISSE, M.; MARTINEZ, C. T. & FERRER, H. F. — A simple volumetric device for preparing stool samples in the cellophane thick-smear technique. *Amer. J. Trop. Med. & Hyg.* 18:553-554, 1969.
7. MARTIN, L. K. & BEAVER, P. C. — Evaluation of Kato thick-smear technique for quantitative diagnosis of helminth infections. *Amer. J. Trop. Med. & Hyg.* 17:382-391, 1968.
8. SCOTT, J. A. & HEADLEE, W. H. — Studies in Egypt on the correction of helminth egg counts data for the size and consistency of stools. *Amer. J. Hyg.* 27:176-195, 1938.
9. STOLL, N. R. & HAUSHEER, W. C. — Concerning two options in dilution egg counting: small drop and displacement. *Amer. J. Hyg. (Suppl.)* 6:134-145, 1926.

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