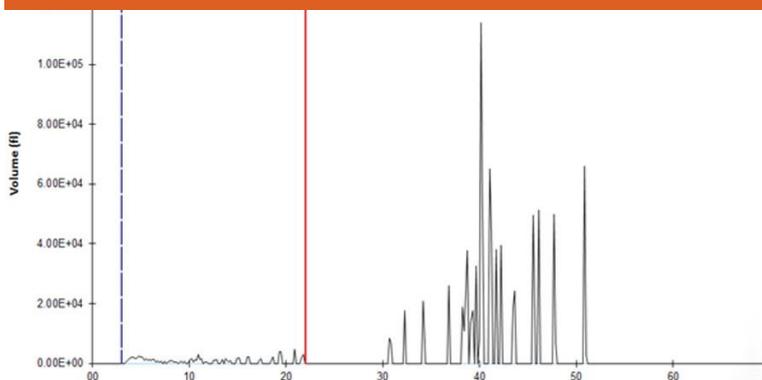


Schistosoma: Egg Quantitation for Drug Discovery

CASY evaluates egg count & size as a readout for compound-induced effects



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• Introduction

The blood fluke *Schistosoma mansoni* causes schistosomiasis, one of the most abundant parasitic neglected tropical diseases. Severe hepatic pathology in patients is induced mainly by worm eggs that get trapped in liver tissue (1).

In basic research, *in vitro* treatment of schistosomes with candidate compounds and quantification of the number of eggs being laid is one important approach for drug candidate discovery. The currently applied microscopic counting of eggs by research groups only allows low-throughput screening. CASY is able to automatically quantify eggs even in low numbers in almost similar accuracy but shorter time, and in addition allows discrimination of normal-sized and abnormal-sized eggs.

• Methods

Sample collection

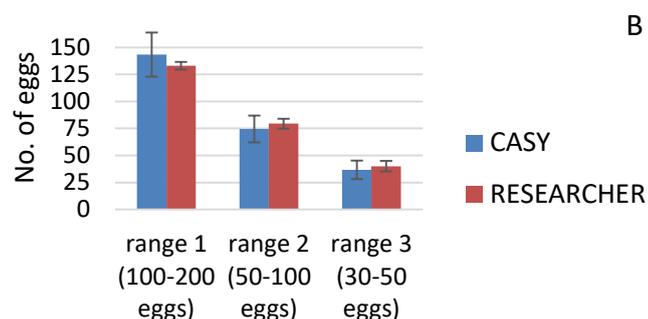
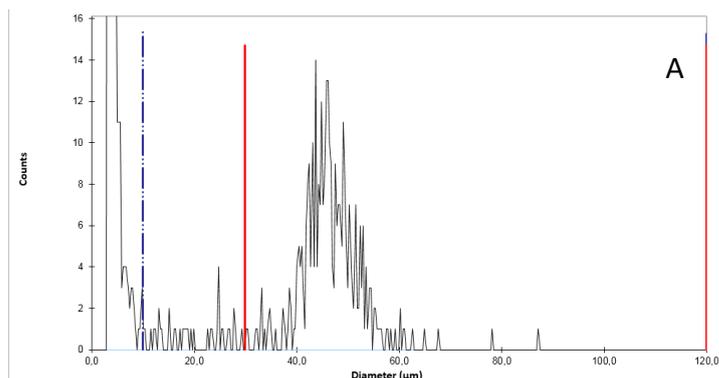
In vitro-laid *S. mansoni* eggs were collected in tissue culture medium (containing 10% FCS), transferred on a 30 µm cellstrainer (Pluriselect) and washed with CASYton to avoid background noise during measurement due to serum components. Egg samples were manually counted using an inverted microscope and then re-measured by CASY. Normal-sized and (compound-induced) abnormal small eggs were manually selected and counted the same way.

CASY analysis

Egg samples were analyzed by CASY (150 µm capillary; size scale: 0-70 µm; sample volume 8-10 x 400 µl; total volume 10 ml) for egg number, egg size and total egg volume.



Fig. 1: Sample preparation. Transfer eggs to cellstrainer, wash twice with 5 ml CASYton and flush eggs into CASYtube with 10 ml CASYton from inverted cellstrainer



Results

Egg counts were adequately quantified even in samples with low total numbers, i.e. as low as 30 eggs per sample (Fig. 2). The mean % deviation from the manual counts obtained by a researcher was 15%.

Egg size was reflected by the peak volume and clearly differed between normal-sized eggs and abnormal small eggs (Fig. 3), which are produced e.g. after compound treatment of schistosomes and can serve as additional readout for compound-induced effects.

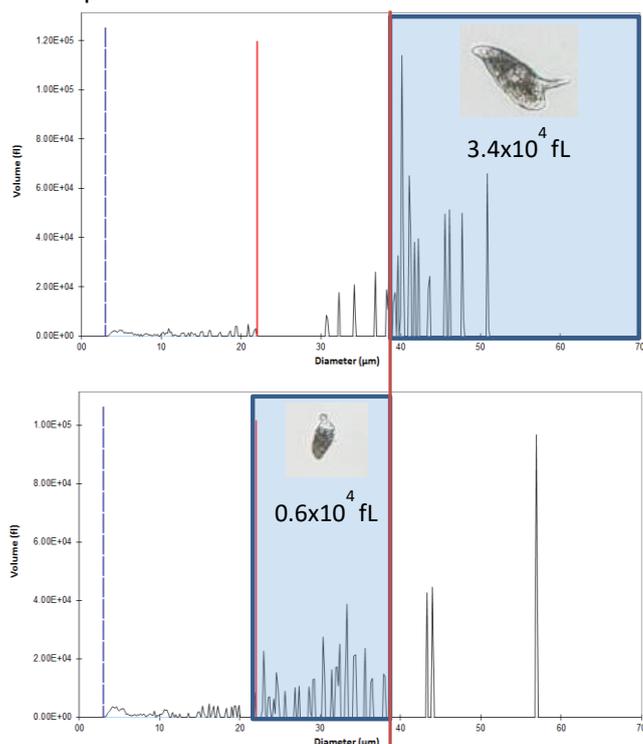


Fig. 3: Quantification of egg size. Normally sized eggs show a peak volume of 3.4×10^4 fL and a peak diameter of $40 \mu\text{m}$, while abnormal smaller eggs (e.g. after compound treatment of schistosomes) show a smaller peak volume of 0.6×10^4 fL and a peak diameter of $23 \mu\text{m}$; few large-diameter peaks might reflect clumps of several eggs. Volume vs. diameter is displayed.

Fig. 2: Quantification of egg numbers. (A) Representative CASY graph displays eggs count vs diameter (μm). (B) Comparison of quantification of different numbers of schistosome eggs (range 1 to 3) by CASY vs. microscopic counting by a researcher. Summary of two experiments with 3-6 replicates, +/- SD.

Conclusion

This study demonstrates CASY's capability to **quantify helminth eggs**, here from the blood fluke *S. mansoni*. **Egg number** even at low counts as well as **egg size** were determined with high accuracy and compared to the commonly used, time-consuming manual counting by a researcher.

CASY **speeds up** compound-screenings in anthelmintics discovery. Furthermore, by the ability to apply increased sample volumes (here: $8 \times 400 \mu\text{l}$), **statistical significant** sample counts come into reach and stabilize study results. Also, egg size can easily be categorized by **automatic volume measurements** of CASY, providing an enhanced statistical basis, compared to manual visual inspection.

It appears to be feasible to also count eggs of other helminth species at least up to a length of $200 \mu\text{m}$, the size of schistosome eggs.

Reference

1. WHO 2018, Schistosomiasis Fact Sheet, retrieved from <http://www.who.int/news-room/fact-sheets/detail/schistosomiasis>

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