

# Practices of Mini-FLOTAC in public health

Laura Rinaldi and Giuseppe Cringoli

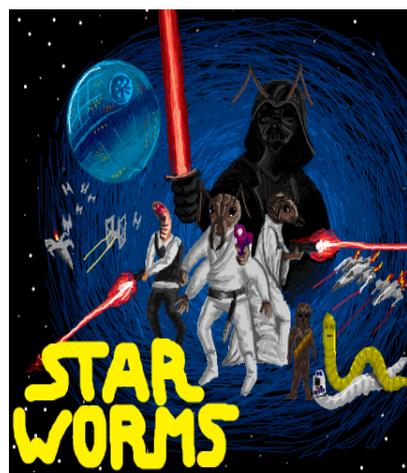
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PI Meeting – STARWORMS  
Gent, Belgium, Feb 23-26<sup>th</sup> 2016

## Outline

- Introduction: the needs
- Mini-FLOTAC
- Mini-FLOTAC & public health
- Discussion



## Introduction



**Veterinary and human parasitology:  
needs of better diagnostics**

*Accurate, simple and  
affordable tests to provide  
results in time for effective  
control measures*

### Diagnostic test

**A** affordable  
**S** sensitive  
**S** specific  
**U** user-friendly  
**R** rapid and **r**obust  
**E** equipment-free  
**D** deliverable

Banoo et al., Nature Reviews Microbiology, 2010

## Introduction



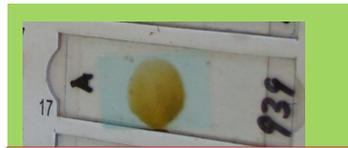
.....a step forward in the fight against  
Soil Transmitted Helminths (STH)



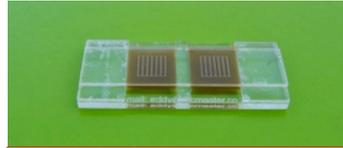
## Introduction

Kato-Katz is the faecal egg count (FEC) technique recommended for the diagnosis of STH. McMaster, FLOTAC and Mini-FLOTAC techniques are used both in veterinary and human parasitology.

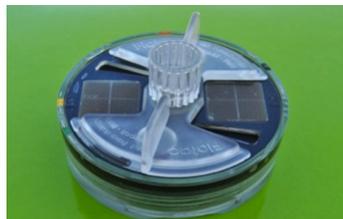
### FEC techniques and analytic sensitivities (EPG)



Kato-Katz  
24 EPG



McMaster  
15-50 EPG



FLOTAC  
1 EPG



Mini-FLOTAC  
5-10 EPG

## Introduction

### Faecal Egg Count (FEC) techniques and performance

FEC Techniques	Diagnostic performance	Technical performance
Kato-Katz	<ul style="list-style-type: none"> <li>➤ A plethora of variants of each FEC technique</li> <li>➤ Standardization of FEC techniques does not exist</li> <li>➤ Diagnostic, research and teaching laboratories often apply their own protocols based on the “lab traditions”</li> </ul>	
McMaster		
FLOTAC		
Mini-FLOTAC		



## Introduction

### Factors often neglected:

- ✓ Accuracy of lab procedures and experience of the laboratory technicians: the human factor!
- ✓ Impact of: fixative used for preservation, duration of faecal preservation before analyses, flotation solution, etc.
- ✓ Helminth eggs should not be considered “inert elements”



## Introduction

### The “FLOTAC strategy” aims at improving the quality of copromicroscopic diagnosis

#### Problems and needs:

- ✓ Absence of a true gold standard (→ modeling)
- ✓ Field and remote diagnosis (→ e-health, m-health)



## Mini-FLOTAC Technique



Mini-FLOTAC is a logical evolution of the FLOTAC technique

The illustration shows a vibrant park scene under a blue sky with white clouds. In the foreground, there are green grass, white daisies, and several cartoon animals including a brown dog, a grey cat, and a black cat. In the middle ground, two children are playing: a girl in a pink dress and a boy in a blue shirt. The background features green trees and a white dove flying in the sky.

### Mini-FLOTAC and Fill-FLOTAC



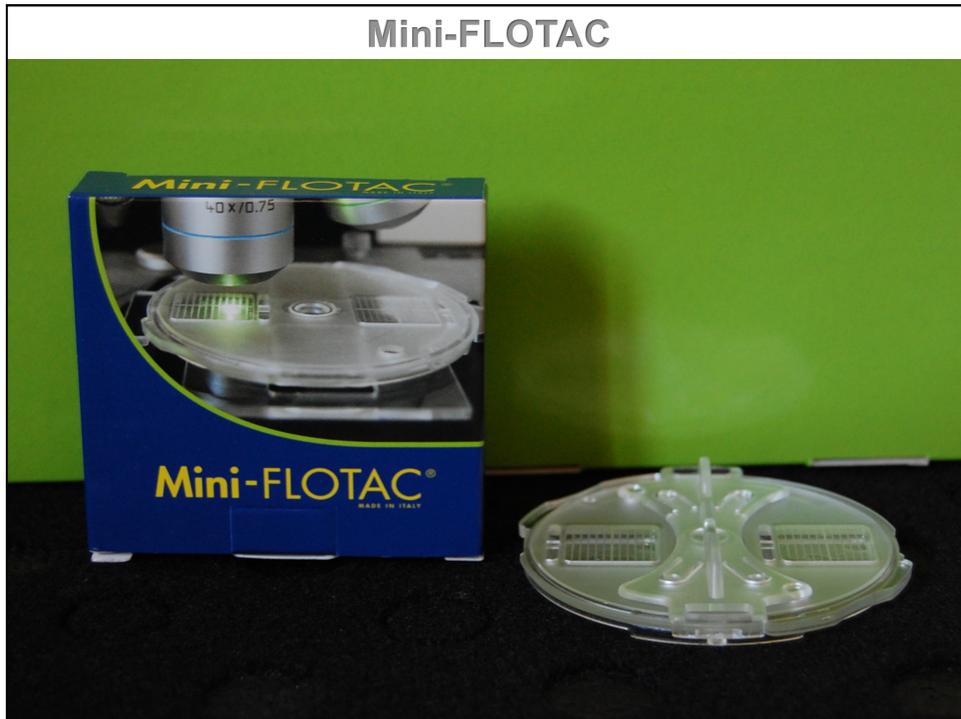
Mini - FLOTAC



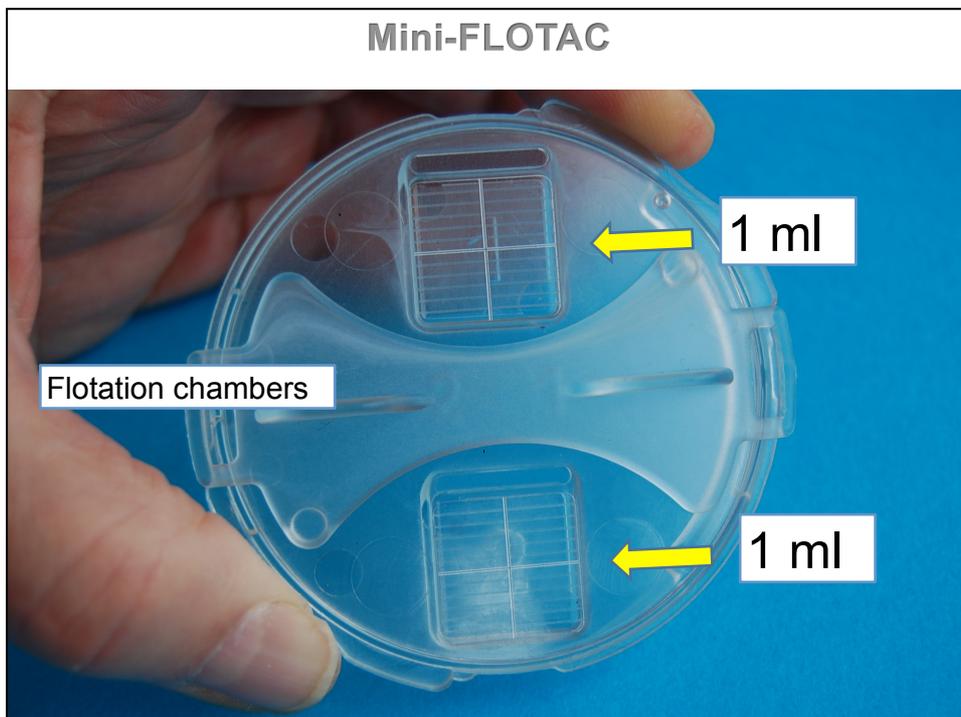
Fill - FLOTAC

- ✓ Closed devices: safe with no risk of contamination for the operator
- ✓ No centrifugation
- ✓ Fresh samples
- ✓ Formalin or any preservative can be added and the samples stored for further testing

### Mini-FLOTAC



### Mini-FLOTAC

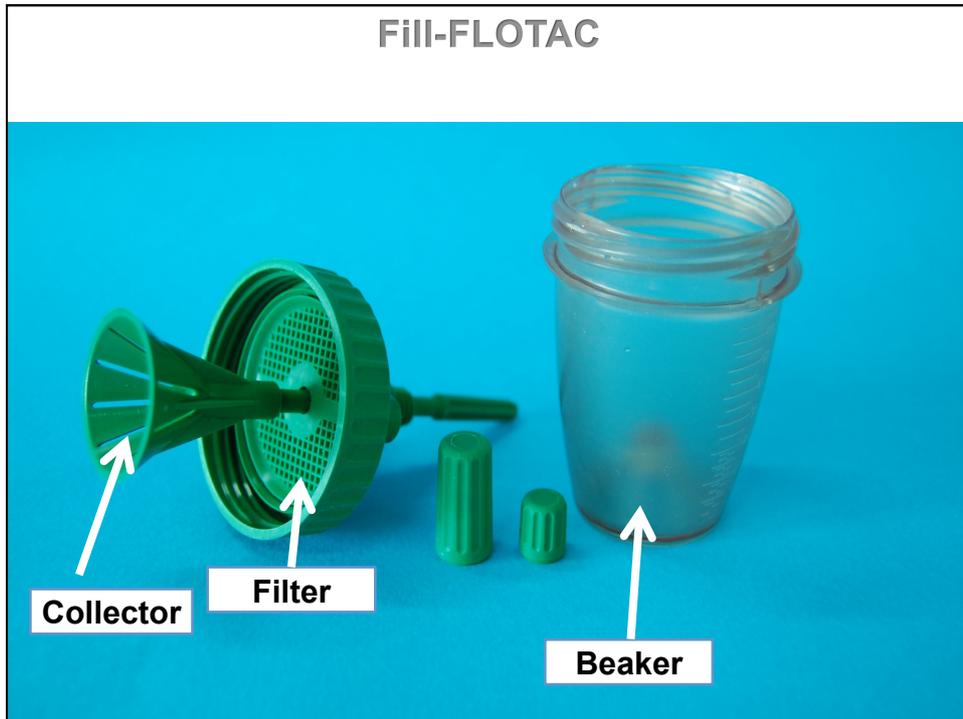


### Fill-FLOTAC



### Fill-FLOTAC



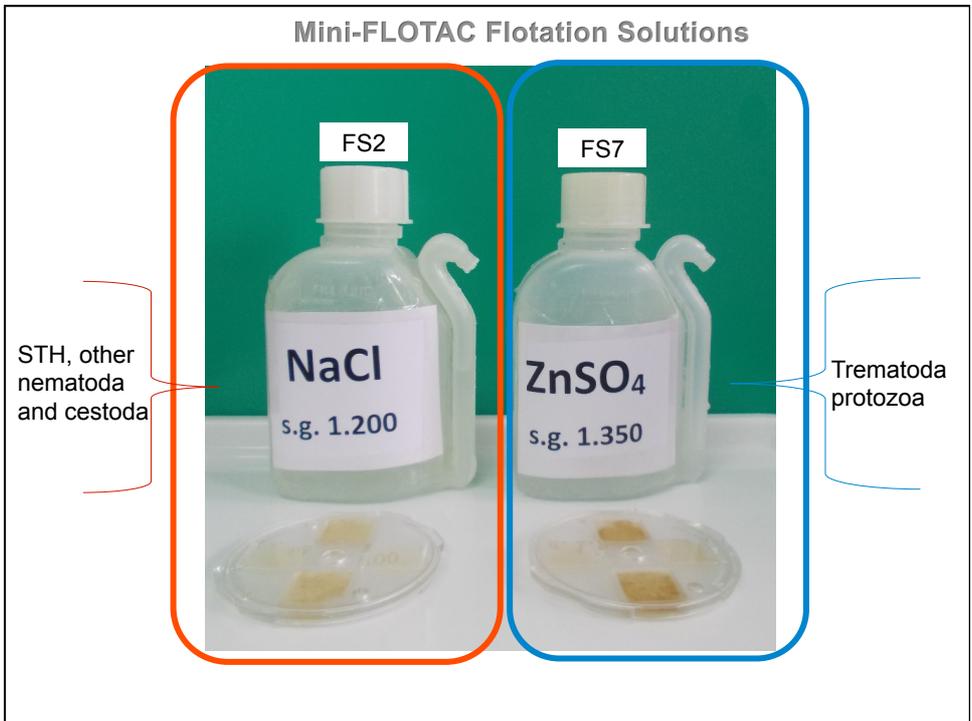


### Mini-FLOTAC technique

Mini-FLOTAC technique on fresh faecal samples

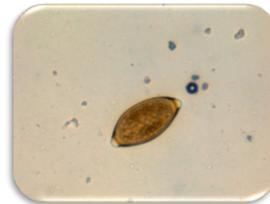
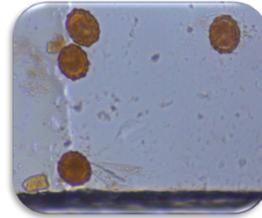
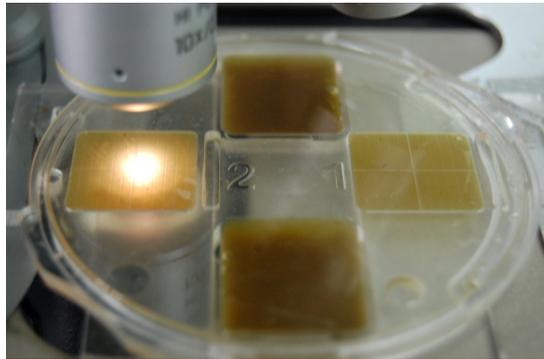
<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>	<b>5</b>

Easy to use also in settings with limited facilities

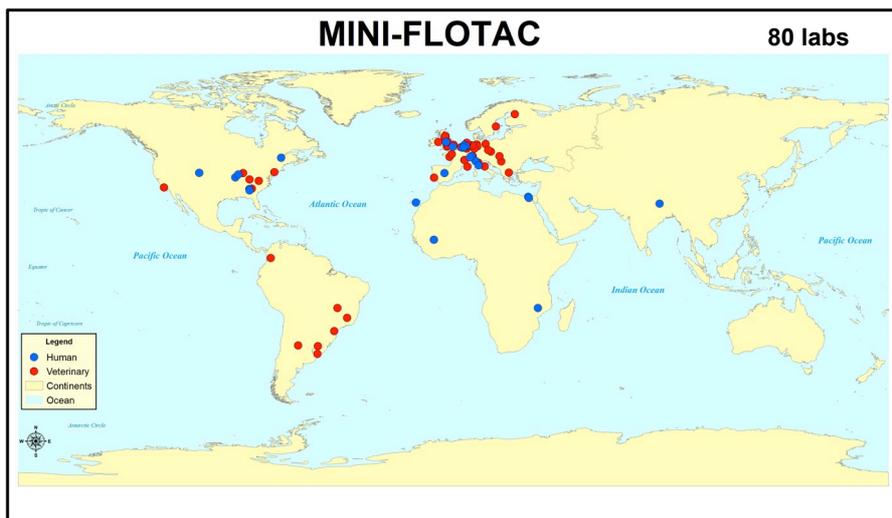


### Mini-FLOTAC

## Mini-FLOTAC under the microscope: STH



## Mini-FLOTAC in public health



### Mini-FLOTAC in public health



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Swiss Tropical and Public Health Institute  
Schweizerisches Tropen- und Public Health-Institut



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### Mini-FLOTAC in public health





Tanzania (Pemba), 2012, 2013, 2015

### Mini-FLOTAC in public health



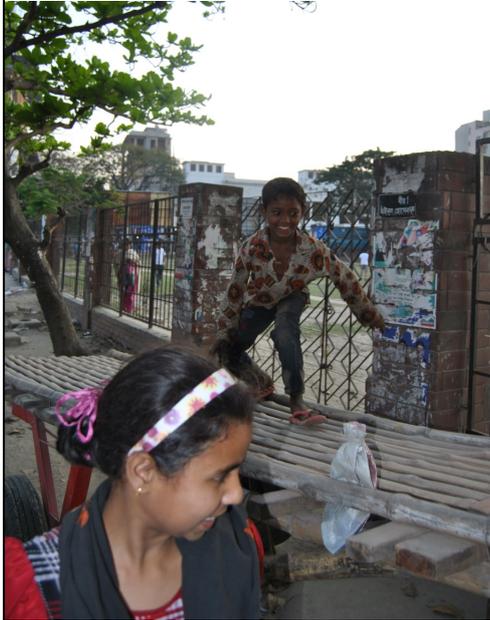
Tanzania (Bukumbi, 2012)

### Mini-FLOTAC in public health



India, 2012

### Mini-FLOTAC in public health

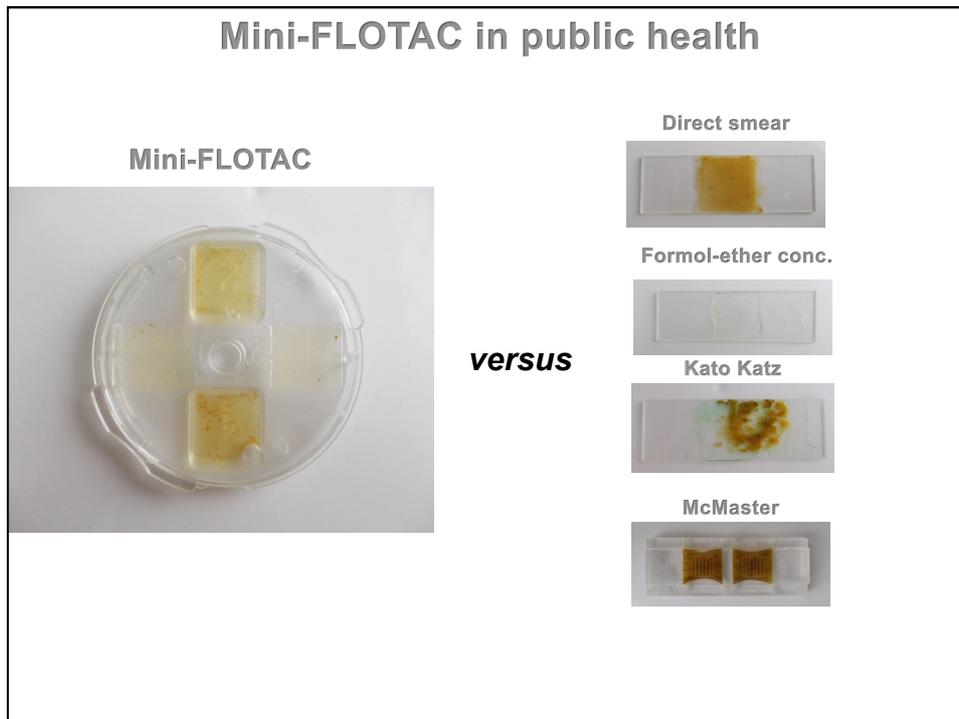


Bangladesh, 2013

### Mini-FLOTAC in public health



Perù, 2013



### Mini-FLOTAC in public health

Settings	n°	FEC Techniques	Helminths investigated
Tanzania (Lake Victoria)	201	Direct smear, Kato-Katz, Mini-FLOTAC	Hookworms, <i>Schistosoma mansoni</i>
India (Dharamsala),	80	Formol-Ether Concentration, Direct smear, Mini-FLOTAC	<i>Ascaris</i> , <i>Trichuris</i> , others
Tanzania (Bukumbi)	100		Hookworms, <i>Schistosoma mansoni</i> , others
India (Dharamsala)	72	Formol-Ether Concentration, Direct smear, Mini-FLOTAC	<i>Ascaris</i> , <i>Trichuris</i> , others
India (Dharamsala)	80		
Argentina	193	McMaster, Kato-Katz, Mini-FLOTAC	STH
Tanzania (Bukumbi, Kigongo, Isamilo and Chole)	251	McMaster, Kato-Katz, Mini-FLOTAC	Hookworms, <i>Trichuris</i> , <i>S. mansoni</i> , others
Tanzania (Pemba)	41	Kato-Katz and Mini-FLOTAC (faeces fixed from 1 to 31 days with 5% formalin)	STH
Kenya (Bungoma)	652	Kato-Katz and Mini-FLOTAC (single and consecutive days)	STH
Bangladesh (100 villages in rural areas)	1630	Only Mini-FLOTAC	STH

OPEN ACCESS Freely available online August 2013 | Volume 7 | Issue 8 | e2344 PLOS NEGLECTED TROPICAL DISEASES

## Mini-FLOTAC, an Innovative Direct Diagnostic Technique for Intestinal Parasitic Infections: Experience from the Field

Beatrice Divina Barda<sup>1\*</sup>, Laura Rinaldi<sup>2</sup>, Davide Ianniello<sup>2</sup>, Henry Zepherine<sup>3</sup>, Fulvio Salvo<sup>4</sup>, Tsetan Sadutshang<sup>5</sup>, Giuseppe Cringoli<sup>2</sup>, Massimo Clementi<sup>1</sup>, Marco Albonico<sup>6</sup>



180 children from two schools:  
 Dharamsala (India) (n = 80)  
 Bukumbi (Tanzania) (n = 100)

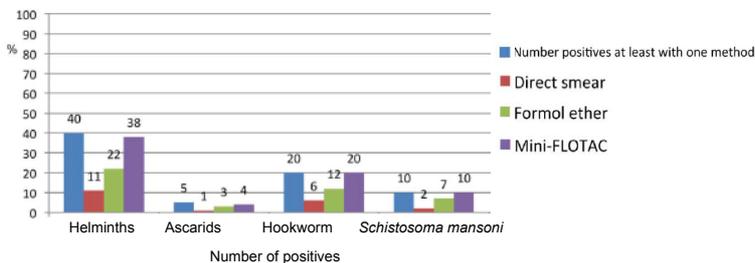
Diagnosis of intestinal parasitic infections with three methods:

- Direct fecal smear
- Formol-ether concentration (FECM)
- Mini-FLOTAC

PLOS Neglected Tropical Diseases | www.plosntds.org Barda et al. August 2013 | Volume 7 | Issue 8 | e2344

### Principal findings

Overall, 72% of the children were positive for any intestinal parasitic infection (helminths and protozoa)



Parasite	Direct smear	Formol ether	Mini-FLOTAC	Number positives at least with one method
Helminths	11	22	38	40
Ascarids	1	3	4	5
Hookworm	6	12	20	20
Schistosoma mansoni	2	7	10	10

Mini-FLOTAC was the most sensitive method for helminth infections (90% mini-FLOTAC, 60% FECM, and 30% direct fecal smear)

Barda et al. *Parasites & Vectors* 2013, **6**:220  
<http://www.parasitesandvectors.com/content/6/1/220>



**RESEARCH** **Open Access**

### Mini-FLOTAC and Kato-Katz: helminth eggs watching on the shore of lake Victoria

Beatrice Barda<sup>1\*</sup>, Henry Zepherine<sup>2</sup>, Laura Rinaldi<sup>2</sup>, Giuseppe Cringoli<sup>2</sup>, Roberto Burioni<sup>1</sup>, Massimo Clementi<sup>1</sup> and Marco Albonico<sup>4</sup>



201 children from two schools in the Mwanza district (Tanzania):  
 Kigongo (n = 90)  
 Isamilo (n = 111)

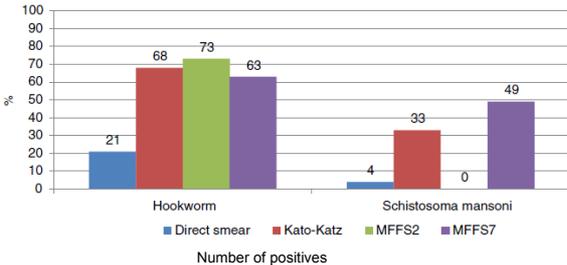
Diagnosis of intestinal parasitic infections with three methods:

- Direct fecal smear
- Kato-Katz
- Mini-FLOTAC

Barda et al. *Parasites & Vectors* 2013, **6**:220

### Principal findings

Overall, 91% of the children were positive for any helminth infections



Parasite	Direct smear	Kato-Katz	MFFS2	MFFS7
Hookworm	21	68	73	63
Schistosoma mansoni	4	33	0	49

Mini-FLOTAC was more sensitive for hookworm (98%) with FS2, and for *S. mansoni* (90%) with FS7 followed by Kato-Katz (91% and 60% respectively) and direct smear (30% and 10% respectively).

## “Freezing” parasites in pre-Himalayan region, Himachal Pradesh: Experience with mini-FLOTAC

Beatrice Barda<sup>a,\*</sup>, Davide Ianniello<sup>b</sup>, Fulvio Salvo<sup>c</sup>, Tsetan Sadutshang<sup>d</sup>, Laura Rinaldi<sup>b</sup>,  
Giuseppe Cringoli<sup>b</sup>, Roberto Burioni<sup>a</sup>, Marco Albonico<sup>e</sup>

### Principal findings

#### 152 subjects (adults and children) examined

**Table 1**  
Prevalence of intestinal parasitic infections with three diagnostic methods: mini-FLOTAC, formol-ether concentration method and direct smear.

	Diagnostic methods									
	Mini-FLOTAC				Formol-ether concentration		Direct smear		Total	
	N. pos F2 (%)	95% CI	N. pos F7 (%)	95% CI	N. pos (%)	95% CI	N. pos (%)	95% CI	N. pos (%)	95% CI
Helminths	11 (7.2)	3.8–12.9	22 (14.5)	9.5–21.3	14 (9.2)	5.3–15.3	9 (5.9)	2.9–11.3	25 (16.4)	11.1–23.5
<i>Ascaris lumbricoides</i>	3 (2.0)	0.5–6.1	16 (10.5)	6.3–18.8	10 (6.6)	3.4–21.1	6 (3.9)	1.6–8.8	17 (11.2)	6.8–17.6
Others	8 (5.3)	2.5–10.5	8 (5.3)	2.5–10.5	5 (3.3)	1.2–7.9	4 (2.6)	0.8–7.0	10 (6.6)	3.4–12.1

Mini-FLOTAC resulted in the detection of higher number of helminths (14.5%) than Formol-ether (9.2%) and direct smear (5.9%)

### Mini-FLOTAC, Kato-Katz and McMaster: three methods, one goal; highlights from north Argentina

Beatrice Barda<sup>1\*</sup>, Pamela Cajal<sup>2</sup>, Eliana Villagran<sup>2</sup>, Ruben Cimino<sup>2</sup>, Marisa Juarez<sup>2</sup>, Alejandro Krolewiecki<sup>2</sup>,  
Laura Rinaldi<sup>3</sup>, Giuseppe Cringoli<sup>3</sup>, Roberto Burioni<sup>1</sup> and Marco Albonico<sup>4</sup>

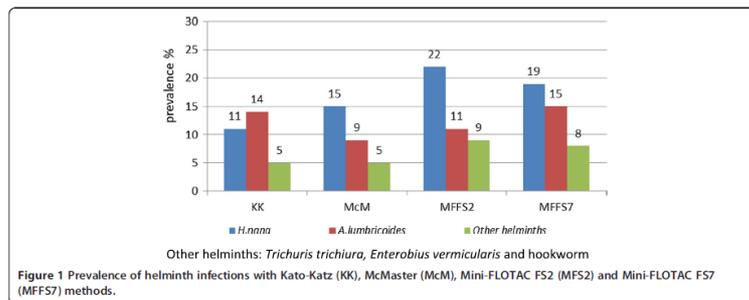


193 schoolchildren from one primary  
school in Oran (Northern Argentina)

Diagnosis of intestinal parasitic  
infections with three methods:

- Kato-Katz
- McMaster
- Mini-FLOTAC

### Principal findings



Mini-FLOTAC was more sensitive than Kato-Katz and McMaster for *H. nana* and as sensitive as Kato-Katz and more sensitive than McMaster for *A. lumbricoides*.

Egg counts differences although relevant, did not reach statistical significance

### Diagnostic Accuracy and Cost-Effectiveness of Alternative Methods for Detection of Soil-Transmitted Helminths in a Post-Treatment Setting in Western Kenya

Liya M. Assefa<sup>1,2</sup>, Thomas Crellen<sup>1,3</sup>, Stella Kepha<sup>2</sup>, Jimmy H. Kihara<sup>3</sup>, Sammy M. Njenga<sup>3</sup>, Rachel L. Pullan<sup>1</sup>, Simon J. Brooker<sup>1</sup>



652 schoolchildren from 18 schools in Bungoma (Kenya)

Diagnosis of STH infections with two methods:

- Kato-Katz
- Mini-FLOTAC

## Principal findings

**Table 2.** Sensitivity and specificity of Kato-Katz and Mini-FLOTAC diagnostic methods for soil-transmitted helminths over single and consecutive day sampling as estimated by latent class analysis.

Test	N samples/groups	Diagnostic error of Kato Katz (95% BCI)		Diagnostic error of Mini-FLOTAC (95% BCI)	
		Sensitivity	Specificity	Sensitivity	Specificity
<b>Single day</b>					
Hookworm	525/18	52.6 (37.8–67.1)	96.5 (92.9–99.3)	47.3 (34.4–60.9)	97.4 (94.5–99.4)
<i>A. lumbricoides</i>	525/18	53.3 (33.9–74.2)	99.0 (97.8–99.8)	50.5 (32.2–72.8)	99.4 (98.3–99.9)
<i>T. trichiura</i>	525/18	52.9 (37.7–72.5)	99.5 (98.6–99.9)	52.5 (37.3–69.7)	99.6 (98.8–99.9)
Any STH	525/18	52.0 (38.5–65.9)	95.5 (90.8–98.9)	49.1 (36.6–63.4)	96.7 (92.2–99.3)
<b>Consecutive day</b>					
Hookworm	132/6	77.6 (61.3–89.2)	93.2 (86.4–98.5)	72.2 (57.0–84.7)	94.9 (89.2–98.8)
<i>A. lumbricoides</i>	132/6	78.2 (56.4–93.3)	97.8 (95.6–99.5)	75.5 (54.1–92.6)	98.7 (96.6–99.7)
<i>T. trichiura</i>	132/6	77.8 (61.2–92.4)	99.0 (97.3–99.8)	77.4 (60.7–90.9)	99.1 (97.6–99.8)
Any STH	132/6	76.9 (62.2–88.3)	91.2 (82.5–97.8)	74.1 (59.8–86.6)	93.5 (85.2–98.5)

Sensitivity was comparable between Kato-Katz and Mini-FLOTAC for STH over a single day (Kato Katz: 52.0%, Mini-FLOTAC: 49.1%) and consecutive days (Kato-Katz: 76.9%, Mini-FLOTAC: 74.1%). Costs were lowest in scenarios using Kato-Katz

## How Long Can Stool Samples Be Fixed for an Accurate Diagnosis of Soil-Transmitted Helminth Infection Using Mini-FLOTAC?

Beatrice Barda<sup>1,2\*</sup>, Marco Albonico<sup>3</sup>, Davide Ianniello<sup>4</sup>, Shaali M. Ame<sup>5</sup>, Jennifer Keiser<sup>1,2</sup>, Benjamin Speich<sup>1,2</sup>, Laura Rinaldi<sup>4</sup>, Giuseppe Cringoli<sup>4</sup>, Roberto Burioni<sup>6</sup>, Antonio Montresor<sup>7</sup>, Jürg Utzinger<sup>2,8</sup>

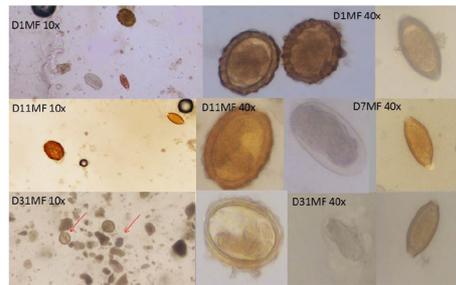
### Mini-FLOTAC task force in Pemba



- ✓ 41 children enrolled
- ✓ Stool samples analyzed at Day 0 with a single Kato-Katz and Mini-FLOTAC
- ✓ 12 aliquots of stool fixed in 5% formalin (1:1) and examined by Mini-FLOTAC up to 31 days after collection.

### Principal findings

- ✓ Kato-Katz and Mini-FLOTAC: similar prevalence estimates for *A. lumbricoides* (85% vs 76%), *T. trichiura* (98% vs 100%), and hookworm (42% vs 51%)
- ✓ Mean EPG according to Kato-Katz and Mini-FLOTAC was 2,075 and 11,679 for *A. lumbricoides*, 1,074 and 1,592 for *T. trichiura*, and 255 and 220 for hookworm.
- ✓ The mean EPG from day 1 to 31 of fixation was stable for *A. lumbricoides* and *T. trichiura*, but gradually declined for hookworm, starting at day 15.



**Fig 4.** Pictures of soil-transmitted helminths at different time points after fixation in formalin. D<sub>1</sub> 10x: *A. lumbricoides*, *T. trichiura*, and hookworm; D<sub>1</sub> 40x: *A. lumbricoides* and *T. trichiura*; D<sub>1</sub> 40x hookworm and *T. trichiura*; D<sub>11</sub> 10x *A. lumbricoides* and *T. trichiura*; D<sub>11</sub> 40x *A. lumbricoides*, D<sub>11</sub> 40x *A. lumbricoides*, D<sub>11</sub> 10x *A. lumbricoides* and hookworm, D<sub>11</sub> 40x *A. lumbricoides*, hookworm, and *T. trichiura*.

### The Interaction of Deworming, Improved Sanitation, and Household Flooring with Soil-Transmitted Helminth Infection in Rural Bangladesh

Jade Benjamin-Chung<sup>1\*</sup>, Arifa Nazneen<sup>2</sup>, Amal K. Halder<sup>2</sup>, Rashidul Haque<sup>2</sup>, Abdullah Siddique<sup>2</sup>, Muhammed Salah Uddin<sup>2</sup>, Kim Kaporc<sup>3</sup>, Benjamin F. Arnold<sup>1</sup>, Alan E. Hubbard<sup>1</sup>, Leanne Unicomb<sup>2</sup>, Stephen P. Luby<sup>4</sup>, David G. Addiss<sup>3</sup>, John M. Colford, Jr.<sup>1</sup>

### Principal findings



1,360 schoolchildren from 100 villages in rural Bangladesh

Diagnosis of STH with Mini-FLOTAC on fixed samples

Prevalence (~40%) associated with deworming, sanitation, and hygiene

## Discussion



- ✓ Mini-FLOTAC techniques are useful for the diagnosis of STH (monitoring and efficacy studies)
- ✓ No competition...but **collaboration, integration and standardization of** diagnostic tools for monitoring the global patterns of effectiveness of drugs and AR in STH control programs



## Discussion

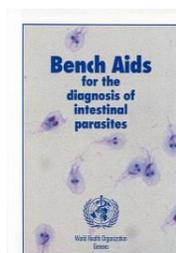
- ✓ Parasitologists and diagnosticians are re-evaluating their attitude of .....  
“it’s only a faecal sample”!



- ✓ Something is changing.....



- ✓ New guidelines ongoing, including SOP for different techniques



## Discussion

- ✓ Ongoing research
- ✓ News: devices in biodegradable plastic



## Discussion

- ✓ Mini-FLOTAC on pooled samples
- ✓ Mini-FLOTAC linked to electronic and mobile health



Mini-FLOTAC in the field



Mini-FLOTAC in the field



Mini-FLOTAC in the field



Mini-FLOTAC in the field



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