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## A Quick Qualitative Test for Identifying Human Blood Meal in Fed Female Mosquitoes

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### Abstract

A one step, double antibody sandwich chromatographic immunoassay test known as Faecal Occult Blood Test, normally used for screening of human patients suspected of colo-rectal carcinoma, was found to be a useful quick qualitative simple test in identifying human blood in engorged abdomens and filter paper smears of fed female mosquitoes. Compared to other specific sensitive tests like PCR, it is simple, field operated, read in only two minutes time and can trace human haemoglobin 72 hrs post mosquito feeding. It is endorsed in epidemiological investigations and as a preliminary screening test in crime scene investigations before the more reliable tests looking for specific DNA are applied.

### Introduction

Most of haematophagous arthropods take blood meals from a variety of hosts. Selection of source from which a blood meal to be taken is still under investigation, though some authors attributed that to host preference and host seeking behaviour (Truls Jensen et al., 1996), availability of host, climatic conditions, resting habits of arthropod, odours of host and visual stimuli (Chaves et al., 2010, CVBD, 2011). The digestive enzymes in the mosquito gut are secreted soon after ingestion of the blood meal and return to base line levels by 60 hrs (Billingsley & Hecker, 1991). Identification of source of blood meal is of paramount importance in understanding feeding behavior of arthropods, important in the study of epidemiology of diseases transmitted by those arthropods and also important in forensic medicine and crime scene investigation. The art of identification of blood meal started early in the 1950s. Direct observation on human baits, blood precipitin test, latex agglutination, haemoglobin crystallization and ELISA were used before the recent advent of PCR technology series of tests identifying DNA (Johansen et al., 2009). PCR tests are more accurate and specific and may detect a blood meal in a mosquito abdomen 60 hrs post feeding (Fornadel & Norris, 2008) and in a sand fly up to 96 hrs post feeding (Abbasi et al., 2009). Most techniques show high degrees of accuracy and specificity, especially PCR though it showed shortness in some instances (Fornadel & Norris, 2008), but all of them are either, time consuming, complicated; needing specialized laboratories and cannot be performed at the field level or costive. A simpler, field operated, less costive and with reasonable degree of accuracy led through observations to a test normally run for diagnosis of

occult blood in human faeces (Simon, 1985). The Faecal Occult Blood Test (FOBT) was introduced to diagnose gastro-intestinal bleeding (Beg et al., 2002). The test is used as an initial routine screening test for diagnosis of colo-rectal carcinomas although may indicate other conditions like ulcers, hemorrhoids or bleeding for some other reasons. There are series of FOBT tests ;Faecal Immunochemical Test(FIT) looking for human globin; Stool Guaiac Test looking for heme; Faecal Porphyrin Quantification Test (HemoQuant) detecting porphyrins in blood and the Faecal DNA Test (The PREGen-Plus) testing DNA. The tests show various degrees of sensitivity and specificity ([http://en.wikipedia.org/wiki/fecal\\_occult\\_blood](http://en.wikipedia.org/wiki/fecal_occult_blood), 20 Sept. 2011, Simon JB., 1998).

The tests are marketed in the form of kits manufactured by different commercial companies. The one step Fecal Occult Blood test (Accurate Rapid Card Test) is a **FIT** rapid one step double antibody sandwich chromatographic immunoassay for qualitative detection of human haemoglobin in faeces. The principle of the test is that the membrane in the kit is pre-coated with the anti-haemoglobin antibody on the test line region. During testing, the specimen mixes with particles coated with anti-haemoglobin and migrates through capillary action to react with the anti-haemoglobin antibody on the membrane to form a coloured line in the test line in case the specimen contains human haemoglobin. The test kit under the name Accurate Rapid Card Test is marketed under the same name in USA, Germany and China.

### Materials and Methods

#### Accurate Rapid Card Test kits

208 Accurate Rapid Card Test kits (China, reagents made in USA) were obtained from the local market. Each kit contains a test device sealed in its pouch and a specimen collection tube with extraction buffer (Fig 1). The device has got two slots; the bottom slot "well S" for applying the specimen buffer mix and the other longitudinal slot having a "T & C" marks on the side for reading of result. A positive test shows two dark lines across the "T & C" side marks (Fig 2 left), a negative shows the line against the "C" mark only (Fig 2 right), while In case of invalid test, no line against the "T or C" is shown.



**Figure 1** Faecal Occult Blood Test showing test kit, pouch and buffer bottle.



**Figure 2** Faecal Occult Blood Test kit showing positive result for human blood (left) and negative result (right).

The 208 Test kits were divided into three groups ; 40, 96 and 72. The first protocol group (40) was for testing the kit on blood obtained from 4 human volunteers (protocol One a), a rabbit, a goat, chicken, a cow, a camel and a horse (protocol one b). The test was repeated four times for each for validation. The

96 kits were for testing on mosquitoes fed on human, rabbit and goat as the second protocol, and the last 72 kits were for testing on wild engorged mosquitoes (protocol three).

### Mosquitoes

Protocol two batch of the 96 mosquitoes for feeding on human, rabbit and goat blood were raised from larvae collected from East Jabal Awlia area and reared in the laboratory in a cage till the adults emerged. Three groups of two day old emerging adults, were separated in small cages covered by net mesh. The first batch was left to feed on human volunteer arm (one of the authors) till visible engorgement, the second was fed on harnessed rabbit ear and the third on goat shaved flank region. 32 out of each cage were randomly selected for further processing. 16 engorged mosquitoes from each cage were removed by aspirator into a plastic cup, sacrificed by chilling in a deep freezer, crushed on a ruled filter paper (Sartorius AG 389, Goettingen, Germany) and left to dry. They were then divided into fours, for testing with the FOBT kits after 1 hr, 24 hrs, 48 hrs and 72hrs. The other 16 engorged mosquitoes were kept alive, divided into four equal groups of 4 each. The first 4 were dissected 1 hr post feeding, the second 24 hrs post feeding, the 3<sup>rd</sup> 48hrs post feeding and the last 72 hrs post feeding. The overall protocol two specimens were 96 (3x32).

The last 72 wild engorged mosquitoes of protocol three were captured from human dwellings of East Jabal Awlia area using aspirator. 24 of which were chilled, dissected to retrieve abdomens and were tested upon. The other engorged 48 were chilled, crushed on 6 filter papers each carrying 8 and left to dry. Specimens in each filter paper were tested, one hour, 24hrs, 48 hrs, 72 hrs, two weeks and four weeks later.

### Processing of Samples

Cap of specimen collection tube of the FOBT kit was unscrewed and the accompanying stick was poked three times in a drop of blood from each of the four human volunteers, dipped into buffer and then the screw was tightened again. The specimen tube was shaken to mix blood with buffer. The tip of the tube was broken and one drop (approximately 50  $\mu$ l) of extraction buffer mix was transferred into well "S" of test device soon after being taken out of the pouch and then left to read in about 2 minutes. The test was repeated in drops of rabbit, goat, cow, chicken, camel and horse blood. For validation, The test for each of the blood samples of human volunteers and the six species of animals was repeated four times bringing a total of 40tests ; 16 human and 24 animal blood samples (protocol one).

Dissected abdomens of mosquitoes in protocol two were individually dropped in the specimen tubes buffer and left to elute at room temperature for 10-20 minutes. Filter paper samples of crushed mosquitoes were individually cut, dropped in buffer and also left to elute at room temperature for 10 minutes. Samples were shaken before testing on kit. A drop from each of the 96 samples was transferred into well "s" of the test kit and results were read in two minutes time. The same procedures were repeated for mosquitoes or filter paper specimens in protocol three.

### Results

The FOBT kits tested on blood of the human volunteers all showed positive in contrast to that of the six animal species samples that were found to be

negative (Table 1& Table 2). The test done on human fed mosquitoes sacrificed 1hr, 24, 48, and 72 hrs post feeding was positive throughout. The filter paper specimens, fresh or stored for 1,24,48 or 72 hrs were all found to be positive too (Table 3).

Abdomens and filter paper specimens of fed mosquitoes on either the rabbit or the goat illustrated negative results throughout (Table 4 & 5). Specimens of abdomens of wild engorged mosquitoes gave 79.17 % positivity (Table 6). The fresh or stored filter paper specimens gave a degree of positivity ranging between 62.5 and 87.5 % with an average of 75% (Table 7).

**Table 1** Results of FOBT kits tested on blood samples taken from the four human volunteers / protocol one (a)

Replicate no.	Volunteer one	Volunteer two	Volunteer Three	Volunteer four	Total
1	+ve	+ve	+ve	+ve	4
2	+ve	+ve	+ve	+ve	4
3	+ve	+ve	+ve	+ve	4
4	+ve	+ve	+ve	+ve	4
<b>Total no.</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>16</b>

**Table 2** Results of FOBT kits tested on blood samples taken from rabbit, goat, cow, camel, horse and chicken / protocol one (b)

Replicate no.	Rabbit blood	Goat blood	cow blood	camel blood	Horse blood	Chicken blood	Total
1	-ve	-ve	-ve	-ve	-ve	-ve	6
2	-ve	-ve	-ve	-ve	-ve	-ve	6
3	-ve	-ve	-ve	-ve	-ve	-ve	6
4	-ve	-ve	-ve	-ve	-ve	-ve	6
<b>Total</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>24</b>

**Table 3** Results of the 32 FOBT kits tested on engorged mosquitoes fed on human volunteer / protocol two

Test Period Post feeding	Dissected engorged abdomens				Crushed mosquitoes on filter papers			
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 1	Replicate 2	Replicate 3	Replicate 4
1hr	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
24hrs	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
48hrs	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
72hrs	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
<b>Total no of tests</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>

**Table 4** Results of the 32 FOBT kits tested on engorged mosquitoes fed on rabbit / protocol two

Test Period Post feeding	Dissected engorged abdomens				Crushed mosquitoes on filter papers			
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 1	Replicate 2	Replicate 3	Replicate 4
1hr	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
24hrs	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
48hrs	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
72hrs	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
<b>Total no of tests</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>

**Table 5** Results of the 32 FOBT kits tested on engorged mosquitoes fed on goat / protocol two

Test Period Post feeding	Dissected engorged abdomens				Crushed mosquitoes on filter papers			
	Replicate 1	Replicate 2	Replicate 3	Replicate 4	Replicate 1	Replicate 2	Replicate 3	Replicate 4
1hr	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
24hrs	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
48hrs	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
72hrs	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
<b>Total</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>	<b>4</b>

**Table 6** Results of FOBT kits tested on dissected abdomens of 24 wild engorged mosquitoes captured in East Jabal Awlia area /protocol three

+ve FOBT		-ve FOBT		Total	
No	%	No	%	No	%
19	79.17	5	20.83	24	100

**Table 7** Results of FOBT kits tested on filter paper crushes of 48 wild engorged mosquitoes / protocol three

Test duration	+ve		-ve		Total
	No	%	No	%	
1hr	5	62.5	3	37.5	8 100
24hrs	7	87.5	1	12.5	8 100
48hrs	7	87.5	1	12.5	8 100
72hrs	5	62.5	3	37.5	8 100
2weeks	6	75	2	25	8 100
4weeks	6	75	2	25	8 100
<b>Total</b>	<b>36</b>	<b>75</b>	<b>12</b>	<b>25</b>	<b>48 100</b>

## Discussion

The quest for a quick, simple and less costive test for identification of human blood in the engorged mosquito or its filter paper smears lead to a test normally run for screening of occult blood passed with faeces of suspected colo-rectal carcinoma patients. The test is known as Faecal Occult Blood Test given the acronym FOBT. It is qualitative and

believed to be accurate with high degree of specificity(98%)and fair sensitivity tracing blood as low as 50 ng/ml or 6µg/gm of faeces (Simon, 1998). This amount of blood is by far less than that imbibed by a mosquito averaging 2.8 µg. Hence, the test is presumed to be even more sensitive in tracing human haemoglobin in engorged mosquitoes. Results of

known human blood from four different volunteers showed positive throughout the four replicates compared with blood of six animal species, on which mosquitoes under normal conditions may feed, which was found negative. The test was also positive when tested on abdomens of mosquitoes fed on human volunteer even 72hours post feeding. This finding is expected since the test was performed during the cold days of the year, Nov.-Dec. where blood digestion is expected to be delayed. Filter paper smears were positive even in the stored specimens at room temperature for up to 72 hrs. Filter paper crushes or engorged abdomens of mosquitoes fed on rabbit or goat tested by FOBT were negative, adding to the fact that the test is specific for human blood even when mixed with digestive enzymes of the mosquito. wild engorged mosquitoes showed less than 100% positivity. This finding of partially negative was expected since there were some animals around the dwellings from which wild mosquitoes might have fed. Filter paper specimens showed varying degrees of positivity in samples tested at different periods post feeding. This finding could mean that filter paper specimens may be fairly tested after four weeks even if stored dry at room temperature. It is not clear yet whether the test may trace extremely small amounts of human blood in mixed meals. Such a limitation or others need to be investigated although even the more sensitive and specific PCR tests do have their limitations (Fornadel & Norris, 2008). From results shown, The FOBT (FIT) screening test could fairly be used as a qualitative, quick (>20 minutes), simple, field operated and moderately cheap test ( $\approx$ 70 cents), for detecting human haemoglobin in either fresh mosquito abdomens or dry filter paper specimens. It could also be used in studying feeding behaviour of other blood sucking arthropods and in epidemiology of diseases transmitted by those arthropods. Furthermore, the test may also be used as a quick preliminary test differentiating human and non-human blood at suspected crime scene investigations before other more specific tests like PCR follow.

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