**Supporting Table S1. Observations with different buffers for mosquito homogenisation**

| Buffer for bead beating | Observation | Considered appropriate |
|---|---|---|
| PBS + sarcosyl-10 | Foam development during beating | No, sarcosyl is better added after homogenisation |
| PBS + protease inhibitor cocktail kit (Thermo Scientific, #78410 ) | Appropriate CSP-ELISA results, appropriate DNA extraction results | No, rapid processing of mosquitoes may obviate the need for protease inhibitor |
| PBS without protease inhibitor cocktail kit (Thermo Scientific, #78410 ) | Appropriate CSP-ELISA results, appropriate DNA extraction results | Yes, sarcosyl should be added to homogenate prior to ELISA, rapid processing of mosquitoes obviate the need for protease inhibitor |

**Supporting Table S2. Experimental infection data CT values by qPCR vs Optical Density in CSP-ELISA.** Mosquitoes infection prevalence for a high and low infectious blood meal was determined by microscopy and compared to OD and positivity in the CS-ELISA and CT-values and positivity in the qPCR. Positive samples in ELISA, defined as the mean optical density of a group of negative blood fed mosquitoes plus three standard deviations and qPCR, qPCR was set at a CT value of 35, are shown in green. Negative samples in red. Samples highlighted in yellow are conflicting, either ELISA or qPCR is positive, the other negative.

| Heat inactivated Microscopy: 0% infection (n=20) | | Low infection Microscopy: 35% infection (n=20) | | High infection Microscopy: 100% infection (n=20) | |
|---|---|---|---|---|---|
| CSP-ELISA OD 0% infection (n=32) | CT qPCR 3% infection (n=32) | CSP-ELISA OD 40.5% infection (n=32) | CT qPCR 37.5% infection (n=32) | CSP-ELISA OD 96.9% infection (n=32) | CT qPCR 100% infection (n=32) |
| 0,065 | 28,58 | 0,056 | - | 3,316 | 29,61 |
| 0,051 | - | 0,47 | 29,20 | 3,284 | 27,15 |
| 0,077 | - | 0,065 | - | 2,735 | 27,36 |
| 0,047 | - | 0,708 | 28,30 | 3,200 | 27,13 |
| 0,074 | - | 0,429 | 28,64 | 2,729 | 28,30 |
| 0,084 | - | 0,058 | - | 2,424 | 27,14 |
| 0,059 | 43,21 | 0,056 | - | 2,425 | 28,89 |
| 0,065 | - | 0,055 | 36,39 | 2,559 | 26,17 |
| 0,051 | - | 0,132 | 36,46 | 1,603 | 27,90 |
| 0,082 | - | 0,574 | 28,21 | 2,268 | 26,78 |
| 0,076 | - | 0,071 | 37,65 | 2,186 | 28,04 |
| 0,063 | - | 0,055 | - | 2,496 | 27,79 |
| 0,054 | - | 0,048 | - | 2,866 | 26,08 |
| 0,054 | 37,50 | 0,709 | 28,23 | 2,315 | 27,58 |
| 0,06 | - | 0,368 | 30,15 | 2,148 | 28,55 |
| 0,052 | 38,92 | 0,06 | 36,53 | 2,446 | 28,17 |
| 0,046 | - | 1,077 | 26,26 | 3,058 | 27,04 |
| 0,068 | 35,75 | 0,068 | 36,46 | 2,34 | 28,20 |
| 0,054 | - | 0,054 | 36,41 | 2,326 | 29,49 |
| 0,063 | - | 0,065 | - | 2,42 | 29,16 |
| 0,045 | 36,38 | 0,582 | 29,93 | 2,98 | 28,29 |
| 0,062 | - | 0,065 | - | 2,039 | 28,12 |
| 0,063 | - | 0,061 | 34,89 | 2,11 | 28,59 |
| 0,051 | - | 0,607 | 27,95 | 2,788 | 27,57 |
| 0,042 | - | 0,059 | 33,26 | 2,981 | 27,46 |
| 0,061 | 36,77 | 0,062 | 34,42 | 0,091 | 28,32 |
| 0,059 | - | 0,986 | 26,88 | 3,003 | 28,43 |
| 0,046 | 37,77 | 0,049 | - | 2,789 | 28,71 |
| 0,051 | 43,20 | 0,057 | 36,32 | 2,361 | 27,45 |
| 0,056 | - | 0,059 | 36,24 | 3,02 | 27,83 |
| 0,052 | 36,71 | 0,501 | 28,50 | 2,535 | 27,57 |
| 0,049 | - | 0,583 | 28,91 | 2,978 | 28,47 |

**Supporting Table S3. Overview of processing time of the four methods compared in seconds per 80 mosquitoes.**

| Method (per 80 mosquitoes) | Time (s) | Total time (s) |
|---|---|---|
| Mosquito dissection + microscopy reading | 7400 | 7400 |
| Preparation of tubes and addition of mosquitoes + Pestle grinding (motor driven) + ELISA (single channel pipette) | 600 + 800 + 2600 | 4000 |
| Preparation of plate and addition of mosquitoes + Bead beating + ELISA (multichannel pipette) | 480 + 100 + 2400 | 2980 |

For each method a total of 80 mosquitoes were processed. Time starts after collecting 80 mosquitoes from each cage.

Microscopy

- Preparation of a slide with mercurochrome (2 mosquitoes per slide), removal of an individual mosquito, dissection and removal of midgut in mercurochrome, discarding carcass.

= Approx. 1 minute per mosquito.

- Storage for 10 minutes in mercurochrome (performed overlapping – so that the five-ten minutes it takes to stain a dissected gut can be filled with further dissections)

= No additional time

- Reading oocysts

= Approx. 30 seconds (appropriate for low intensity infections, >1 minute for high intensity infections).

- Additional time to copy up results

= 15 minutes

**80 mosquitoes in approx. 124 minutes**

Pestle grinding (motor) or bead-beating

Label tubes and add mosquitoes to eppendorf, or label plate and add mosquitoes to wells

= 4 minutes or 1.5 minutes

Pestle grinding (motor driven) = 7 seconds per mosquito

Bead beating = 10 seconds per plate (80 mosquitoes)

CSP-ELISA

Preparing coating and buffers

= 10 minutes

Coating ELISA plate, adding sample (3 hours incubation)

= 5 minutes (incubation = no additional time)

Washing plate 3 times + addition of blocking buffer

= 10 minutes

Incubation 1 hour

= no additional time

Preparing standard curve of recombinant CSP

= 5 minutes

Plate incubation overnight at 4°C

= no additional time

Adding monoclonal and three hours incubation

= 1 minutes (incubation = no additional time)

Washing plate 4 times

= 10 minutes

Adding substrate

= 1 minutes

Stop reaction

= 1 minutes

Read absorbance

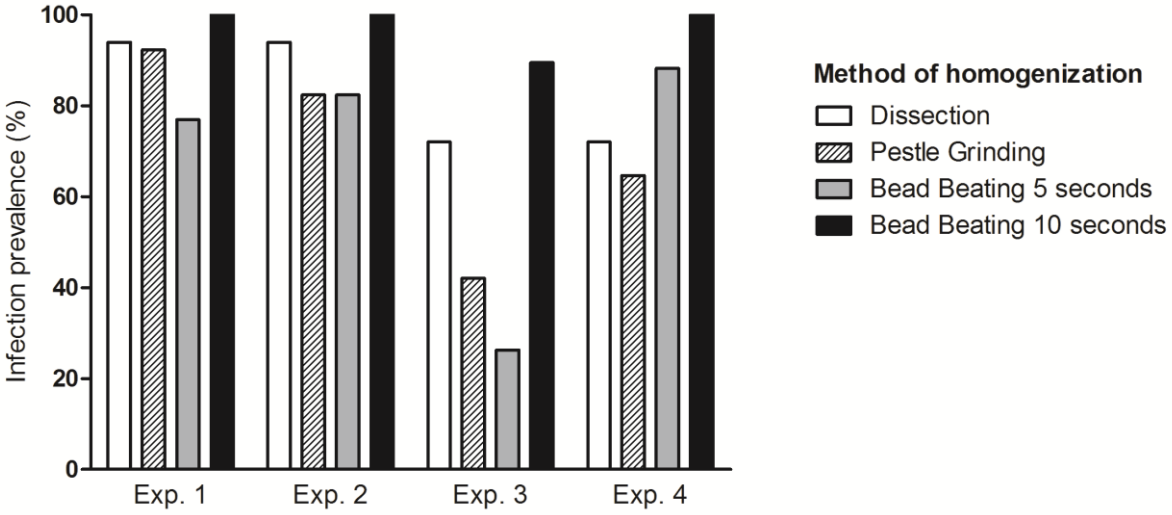
= 2 minutes

**80 mosquitoes in approx. 45 minutes**

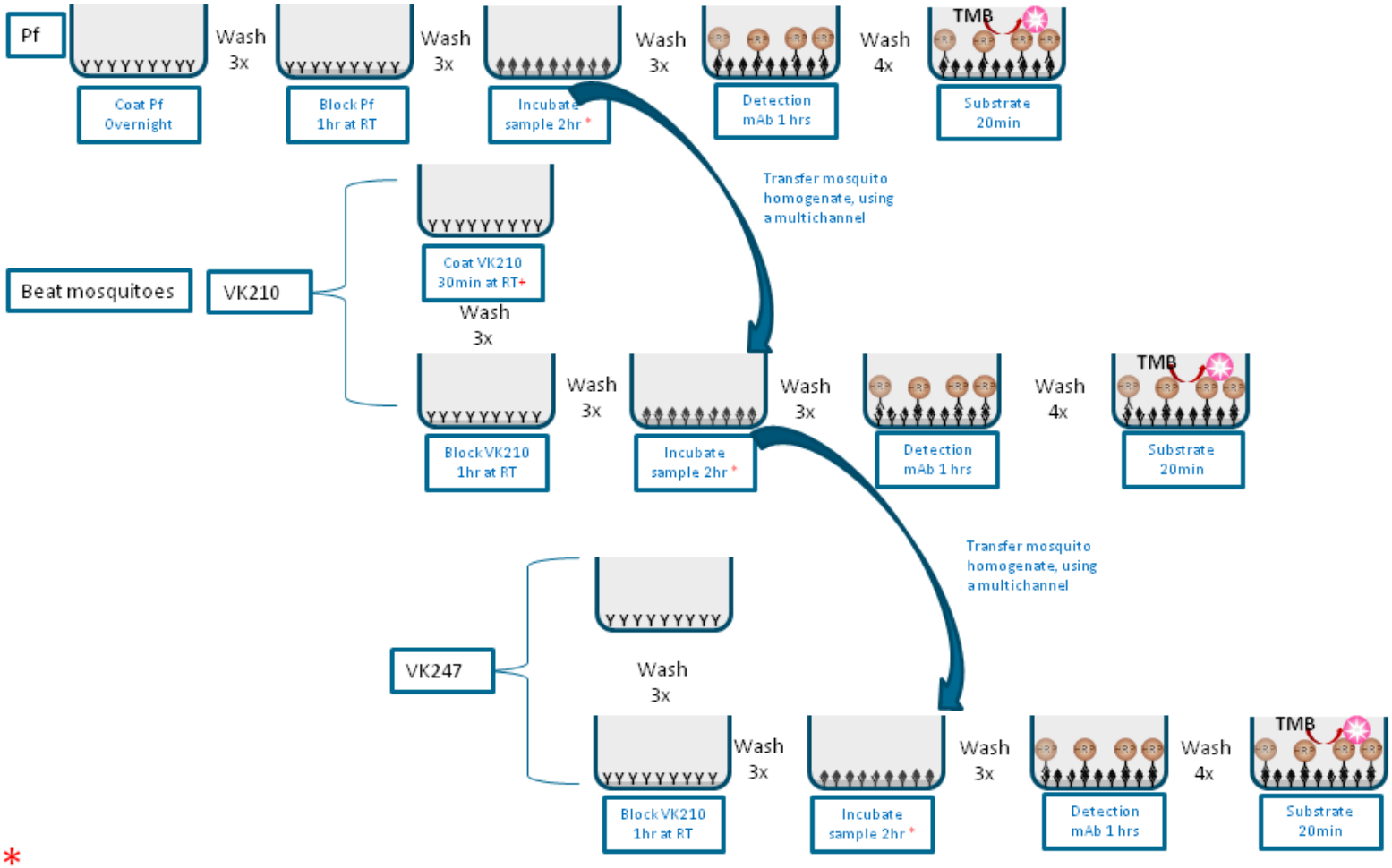
When bead beating was performed in a single 96-deepwell plate and a multichannel pipette was used to transfer material, an ~ 2.5 fold increase in throughput, compared to traditional microscopic evaluation can be achieved.

**Supporting Figure S1. The effect of homogenization method on estimated infection prevalence.**

In four independent experiments 69, 81, 87 and 81 were available to test homogenization methods. Thirty mosquitoes were processed for infection detection by microscopy on day 7 post infection (open bars). Remaining mosquitoes were divided in 3 batches with equal mosquito numbers (i.e. 13, 17, 19, 13 per batch for experiments 1, 2, 3 and 4, respectively) and processed by pestle grinding (hatched bars) and bead beating for 5 seconds (grey bars) or 10 seconds (black bars). Bead beating for 10 seconds consistently gave similar or higher infection prevalence estimates compared to bead beating for 5 seconds. The infection prevalence by 10 second bead beating was not statistically significantly different from the microscopy estimate, differences between these conditions probably reflecting variation in infection prevalences in batches of mosquitoes.



**Supporting Figure S2. Processing of mosquitoes by CS-ELISA when tested for both *P. falciparum* and *P. vivax***




* Make sure that the 'labeled antibody solution' was prepared before the ' sample incubation period' of the Pf ends and the same for the VK210 plate is finished.

\+ do this right before incubating the previous sample