**Turn around time – what you gain and what you loose**

Comments and discussions concerning specimen turn-around-time [TAT] by the Arbovirus Laboratory of the Center for Vectorborne Diseases [CVEC] prompted to us to critically assess our TAT during 2004 and make improvements for 2005. Recent completion of temperature studies now allow the estimation of WNV growth within *Cx. tarsalis* females maintained at different temperatures and therefore the time from infection to viral detection by different diagnostic methods. This allows sensitivity and TAT to be considered within the context of virus transmission dynamics.

**Turn around time during 2004**. The actual time at CVEC for testing bird tissues from receipt to reporting was estimated during May [n = 91 birds], July [n = 95 birds] and Sep [n = 67] 2004 for randomly selected samples [Fig. 1]. Means for these three periods [±95% CL] were 3.5±0.3, 7.0±0.4 and 6.5±0.2 days, respectively. In May bird tissues were received on Tuesday, tested on Wednesday and reported on Friday so that TAT did not include weekends. During July

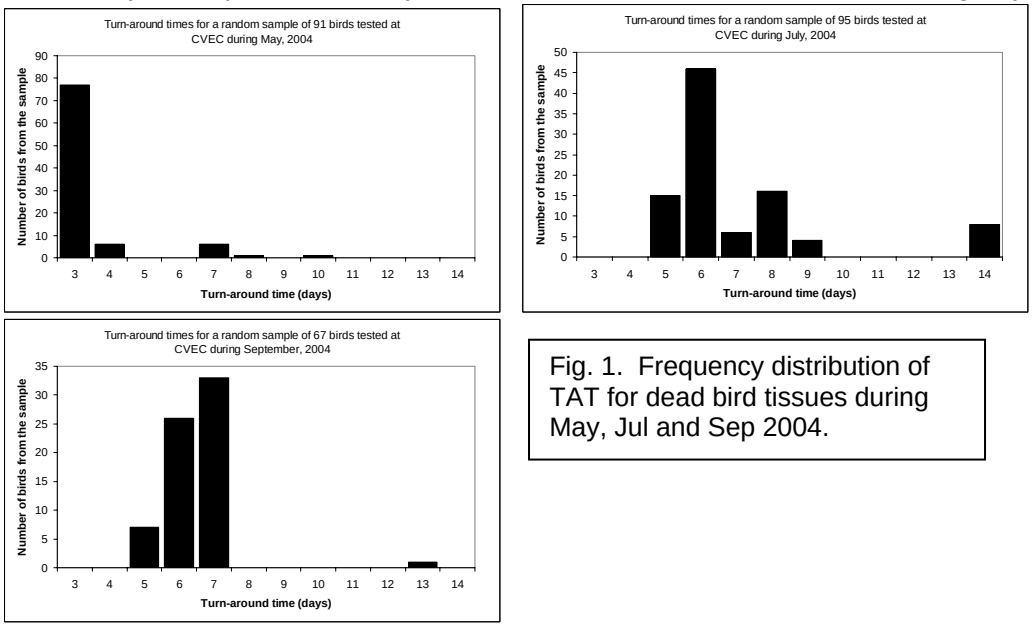


Fig. 1. Frequency distribution of TAT for dead bird tissues during May, Jul and Sep 2004.

and September, bird tissues received on Fridays were tested the following Monday and Tuesday so that TAT now included the weekends. Delays in all months were due to some misfiling of specimens, resolving border-line positives that required further testing to assure correct determination, the Taqman being inoperable for 3 days in mid July, and delays related to a weekly reporting schedule. However, changes in the testing protocol allowed us to receive CAHFS necropsy tissues on Friday and then test them the following week before most mosquito pools were received thereby allowing faster TAT for pools received before Wednesdays.

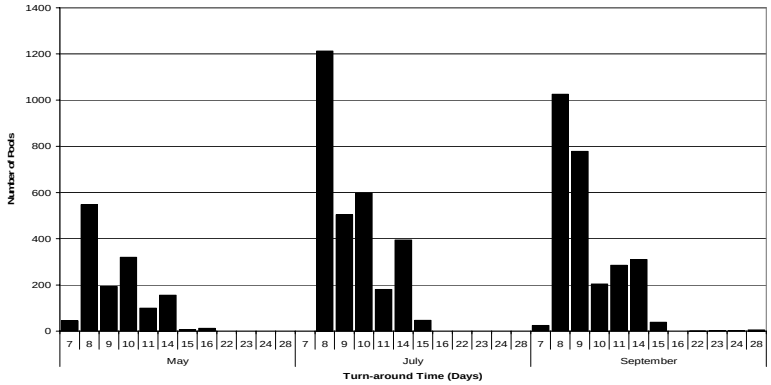


Fig. 2. TAT for mosquito pools tested at CVEC during May [n = 1,382], Jul [n = 2,938] and Sep [n = 2,678] 2004.

We used a similar analysis to determine TAT for mosquito pools during the same three month period, except that measures were calculated for every pool submitted [Fig. 2]. Means for the three representative months were 9.6, 9.7 and 9.5 days, respectively, and TAT did not differ between negative and positive pools. Outliers taking longer than the means frequently were positives that required additional testing that were reported during the following weekly bulletin.

The "2004 battle hardened" and well-trained CVEC staff have made adjustments to enhance TAT during 2005. The addition of a second Taqman for RT-PCR provided by supplemental CDC funding will significantly shorten TAT and provide a back-up. Enhanced data management will facilitate more frequent reporting and should enhance TAT. Bird tissues arriving by Fri will be tested on Mon-Tues and reported by Wed [i.e., 6 days] including the 2 day weekend. For mosquito pools arriving at CVEC between Fri and the following Wed, we can commit to a maximum turn-around time of 7 days. In some cases TAT may be as fast as 3 days for pools arriving at CVEC by Wednesday morning. This high throughput, sensitive assay system now has one of the fastest TATs for any laboratory in the country considering the modest charge of $18/pool and the limited staff of 3.5 individuals.

**Virus dynamics and TAT**. Although laboratory TAT is critical, it also is useful to consider these data in the context of WNV transmission dynamics and sampling [Fig. 3]. American crows

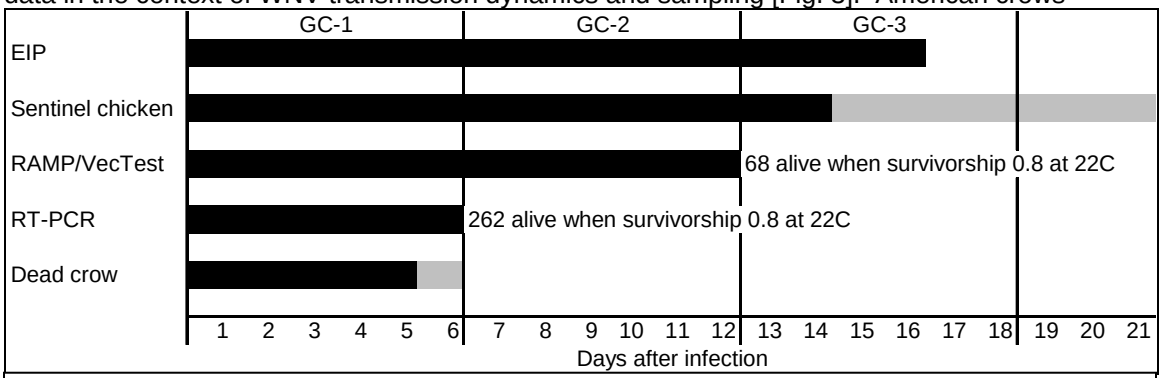


Fig. 3. Time from infection on day 0 until virus can be detected. Data for mosquitoes uses 22C [72F], because sensitivity of early warning systems are more critical during spring. GC = duration of the gonotrophic cycles 1 to 4, EIP is the duration of the extrinsic incubation period from infection to transmission .

die when acutely ill about 5 days after infection on day 0 (Brault et al. 2005). Other species such as House finches and House sparrows may take 7-9 days (Reisen et al. 2005), whereas raptors can take up to 3 weeks. This would be about the same time at 22C [71 F or spring temperatures] as a mosquito infected while taking its first blood meal on day 0 and returning to

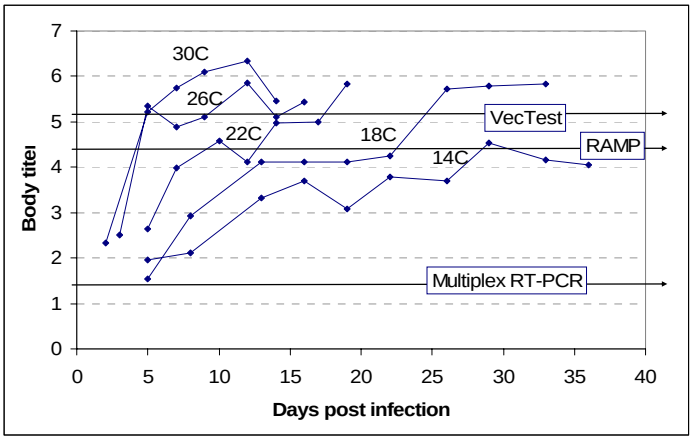


Fig. 4. Quantity of WNV in $\log_{10}$ PFU in *Cx. tarsalis* females plotted as a function of days held at 5 temperatures. Shown are the approximate sensitivities of VecTest, RAMP and RT-PCR assays [E.N. Green unpublished].

take a second blood meal approximately 6 days later. This is the first time a potentially infected mosquito would be collected by dry ice-baited traps. At this time mosquito body titers average ca. 2.6 $\log_{10}$ plaque forming units [PFU] of WNV [Fig. 4] which can be readily detected by the CVEC multiplex RT-PCR assay or by Vero cell plaque assays, but not by the not by the RAMP or VecTest which require >4.3 $\log_{10}$ PFU, respectively [E.N. Green, unpubl.]. Females with body titers >4 $\log_{10}$ PFU were not detected until 9 days post infection, but these females would not be collected until host-seeking at the start of the 3rd gonotrophic cycle at 12 days of age. Considering survivorship to be 0.8 per day (Reisen et al. 1992; Reisen et al. 1995), ca. 262 females of a cohort of 1,000 infected at day 0 would be alive after 6 days, but only 68 [or 26% of those alive at 6 days] would be still alive after 12 days. Therefore, to maintain comparable sensitivity to detect virus presence within the mosquito population, an agency would have to collect and test 4 times more mosquito pools if these were tested by the RAMP or VecTest than they would having mosquitoes tested by the RT-PCR method. These differences became less dramatic at hot summer temperatures, but were worse at cooler spring temperatures that approached the growth threshold of WNV.

Sentinel chickens require ca. 12 days to develop sufficient antibody titer for detection by enzyme immunoassay. If infected during day 1 or 2 of the 2 week bleeding cycle then birds would test positive on the next bleeding; however, if infection came on day 7 of the bleeding cycle, then seroconversion would be detected until day 21 and so on.

Interestingly, at 22C the duration of the extrinsic incubation period or the time from the infectious blood meal until median population transmission was 16 days. This estimate decreased to 6 days at a constant temperature of 30C.

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