DETECTION OF WEST NILE VIRUS ELIZABETH B. KAUFFMAN . [ET AL.] pdf

1: West Nile virus : methods and protocols in SearchWorks catalog

West Nile virus (WNV) belongs to the family www.enganchecubano.com virion contains single-stranded, positive-sense RNA approximately 11 kb in length. In the summer and fall of , an outbreak of WNV infection in the northeastern United States was responsible for 62 human cases, including seven deaths.

See details in Materials and Methods. The sequencing results confirmed the identity of the amplicons as WNV. On the basis of the results described above, we chose 8, 0. Figure 2C presents the results of a representative assay and shows the results for one positive lane 3 and one negative lane 2 mosquito pool and one strongly positive bird sample lane 4. Nested PCR increases the sensitivity. This was expected because the two assays had similar sensitivities see above. A more sensitive assay was required to verify the results for samples with discrepancies. As shown in Fig. Commercial RNA extraction kits are efficient compared to the traditional phenolchloroform extraction method, but they still require manual operation and thus remain a bottleneck for surveillance. Therefore, the use of an automated RNA extraction system was essential to increase sample throughput. At each level of spiked virus, similar CT values were obtained for samples from which RNA was extracted by use of either the ABI Prism workstation or RNeasy, indicating similar recovery rates by the two extraction methods. No difference in viral RNA recovery was observed for the kidney, heart, brain, and spleen tissues. These results indicated that no tissue-specific factors affected the RNA extraction by either method. Similar results were obtained by two independent experiments. Figure 3 shows the autographs of the amplified products analyzed on agarose gels stained with ethidium bromide. Similar intensities of the amplified products were observed by either extraction method at each level of spiked virus. Figures 3A and B show the results for samples extracted from kidney and heart tissues, respectively. Similar results were obtained with brain and spleen tissues data not shown. Furthermore, the ABI Prism workstation dramatically increased the throughput of the procedure. Up to 96 samples could be processed in approximately 1 h by the robotic process, whereas it takes a trained technician approximately 4 h to process the same number of samples by the RNeasy method. For confirmatory purposes, the positive samples were then subjected to a secondary assay with primer-probe set 2 or 3 Table 1. Samples that tested positive by both assays were considered confirmed positives. Since primer-probe sets 2 and 3 are less sensitive than set 1, it was expected that some samples with low levels of WNV-specific RNA from the initial screen would be negative by the confirmatory assay. A portion of the specimens that were not confirmed were analyzed further by the nested PCR assay. During surveillance in the year , all the samples were processed by the RNeasy procedure. However, with the introduction of the high-throughput ABI Prism workstation, laboratory capacity for future surveillance can be significantly increased. A and B Samples extracted from kidney and heart tissues, respectively. Lanes 1, bp markers. PCR, an additional hour of trained technician time can be saved. Samples with or without spiked viruses 1, PFU per sample were processed in neighboring wells. The results showed no cross-well contamination on the well plate during the robotic extraction data not shown. In addition, we tested the reproducibilities of tests with the robotic apparatus by extracting RNA from similar samples several times and quantitating the RNA recovery. The data showed consistent RNA recovery, with little variation data not shown. Streamlined viral RNA detection procedure. Because of its highest sensitivity, primer-probe set 1 Table 1 was used in the initial The recent outbreaks of WNV in the northeastern United States and certain regions of Europe and the Middle East have made it essential to develop an efficient procedure for surveillance. High-throughput detection of WNV in specimens submitted to the laboratory is essential for surveillance because i the number of samples is very large and ii the diagnosis usually has a demanding timeline since the results will have an immediate effect on intervention decisions such as mosquito pesticide spraying. Nucleic acid-based techniques, especially RT-PCR, have the advantages of speed, specificity, and sensitivity. The combination of the ABI Prism workstation and real-time RT-PCR automated the complete procedure for nucleic acid extraction, amplification, and product detection and, therefore, dramatically

increased the throughput and the capacity of diagnosis. This procedure allows testing of 96 samples in approximately 4. A nested PCR assay was developed to further improve the detection limit by approximately fold. A key component of high-throughput diagnostics is RNA extraction. These two systems are based on the silica membrane purification method; the ABI Prism workstation uses a novel extraction mechanism unpublished data. The ABI Prism workstation preferentially captures RNA on a glass-fiber membrane with an electronically controlled vacuum filtration mechanism. Genomic DNA is retained in solution and passes through the glass-fiber membrane with virtually no retention. In contrast, the ABI Prism workstation is enclosed in its own safety cabinet, thus preventing potential aerosol exposure and virus contamination. A second key component of high-throughput nucleic acid detection is real-time RT-PCR, which incorporates amplification and detection into one step We routinely tested positive specimens using two primer-probe sets in two separate reactions. To further improve the throughput and reduce the cost of the assay, multiplex real-time RT-PCR assays that combine the two primer-probe sets in one reaction tube are under development. Another advantage of real-time RT-PCR is the decreased risk of contamination of the laboratory with PCR product because the reaction mixture is discarded without opening the tube at the end of the amplification. The sensitivity of the primer-probe sets was 50 to molecules. We found a similar level of sensitivity 0. An alternative technology, Molecular Beacon 24 , exists, but there have been no reports on its use for WNV detection. A recent comparison of the TaqMan and Molecular Beacon technologies showed that they have similar sensitivities for the detection of DNA in clinical samples Several factors could explain the improvement in sensitivity: We found that the addition of Q solution, an additive reagent from the One-Step RT-PCR kit, to the reaction mixture improved the sensitivity of the assay by more than fold. This may be because Q solution changes the melting behavior of nucleic acids and thus increased the amplification efficiency. However, we found that the addition of Q solution did not increase the amplification efficiency in assays targeting other regions of the WNV genome unpublished data. We also found that a high primer concentration up to 0. In order to maintain the consistency of the standard RT-PCR, we strictly controlled the conditions of the assay to minimize experimental variation. These included the use of freshly made gels, standard ethidium bromide concentrations, and standard electrophoresis times. Freshly thawed aliquots of control standards were used throughout the surveillance season. Due to its J. Both RT-PCR and nested PCR were performed in individual tubes rather than strip tubes because contamination can easily occur between the strip tubes during assembly of the reaction. NucliSens uses the electrochemiluminescence method for sensitive detection and yields clear-cut positive and negative signals. This is in contrast to the electrophoresis detection method, which frequently provides equivocal results when small amounts of amplified products are analyzed. We are very grateful to Paul Masters for help during the development of the assays. Detection of West Nile virus sequences in cerebrospinal fluid. Flavivirus genome organization, expression, and replication. Nucleic acid sequence-based amplification. Zwischenmolekulare energy-wanderung und fluoreszenz. Epidemiology of West Nile infections in the South of France, p. In V Bardos ed. In Principles of fluorescent spectroscopy. Plenum Press, New York, N. Origin of the West Nile virus responsible for an outbreak of encephalitis in the northeastern United States. Rapid detection of West Nile virus from human clinical specimens, field-collected mosquitoes, and avian samples by a TaqMan reverse transcriptase-PCR assay. Isolation from human sera in Egypt of a virus apparently identical to West Nile virus. Raven Press, New York, N. Louis encephalitis viruses by Culex tarsalis Diptera: A neurotropic virus isolated from the blood of a native of Uganda. Homogeneous scoring of single-nucleotide polymorphisms: A study of the ecology of West Nile virus in Egypt. West Nile encephalitis epidemic in southeastern Romania.

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2: Virus Detection Protocols for West Nile Virus in Vertebrate and - www.enganchecubano.com

The recent outbreaks of West Nile virus (WNV) in the northeastern United States and other regions of the world have made it essential to develop an efficient protocol for surveillance of WNV.

In the present report, we Downloaded from http: The procedure analyzed 96 samples in approximately 4. The standard assay can be reliably used to test a small number of samples or to confirm previous test results. The results of the study demonstrated for the first time that the use of an automated system for the purpose of large-scale viral RNA surveillance dramatically increased the speed and efficiency of sample throughput for diagnosis. West Nile virus WNV belongs to the family Flaviviridae, Transmission predominantly involves mosquitoes of the Culex which comprises over 70 viruses. Most flaviviruses are arthro- genus and wild birds as the reservoir host. In regions of ende- pod borne and are transmitted to vertebrates by infected mos- micity, many infections are often asymptomatic or cause mild quito or tick vectors. Many flaviviruses are significant human disease However, human epidemics of severe disease pathogens, including WNV, yellow fever virus, four serotypes were reported in Israel Centers for Disease Control and Pre- of dengue virus, Japanese encephalitis virus, Murray Valley vention [CDC] web pages, accessed 29 October , France encephalitis virus, and tick-borne encephalitis virus Like 10 , Romania 23 , and Russia 7, 9. Both eastern United States sickened more than 60 people and termini of the genomic RNA contain sequences that do not caused 7 deaths 8. Extensive mortality in crows Corvus spe- encode viral proteins, known as the 59 untranslated region cies and the deaths of several exotic birds at a zoologic park in UTR and the 39 UTR. The genomic RNA of flaviviruses the same geographic area were concurrently observed Since the outbreak, intensive capsid [C], premembrane [prM] or membrane [M], and en- surveillance has been instituted to monitor the spread of WNV velope [E] proteins and seven nonstructural NS1, NS2a, among mosquitoes, birds, and other vertebrates. Flavivirus lance results provided direct evidence of WNV activity and virions are spherical in shape with a diameter of 40 to 60 nm. Accumu- and genomic RNA and is surrounded by a lipid bilayer in which lated data on viral infection among various species of mosqui- the viral envelope and membrane proteins are embedded 4. WNV was originally isolated in from the blood of a toes and birds as well as other vertebrates e. The virus is widely distributed throughout Africa, the Confronted with increasing numbers of samples for surveil- Middle East, part of Europe, Russia, India, and Indonesia. The procedure involves extraction of RNA from Fax: The RT-PCR product containing the most commercial kits use a silica gel-based membrane to se- genomic sequence from nucleotides nt to included the coding regions lectively bind RNA or DNA under differently formulated so- of the complete prM or M and E proteins and the N-terminal region of the NS1 lutions. Although the standardized commercial kits are faster protein. The extensive surveillance season. In this report we describe a high-throughput de- enzyme digestion and DNA sequencing. The results of the present lowed by ethanol precipitation. Briefly, study demonstrate for the first time that an automated system 1 mg of plasmid was used in a ml reaction mixture. The transcription reaction can be used for large-scale viral surveillance. The meth- extraction and ethanol precipitation and quantitated with a spectrophotometer. African green monkey kidney Vero cells were grown in Biosystems. WNV flamingo was kindly provided by the Na- mM. Because of the difference in elution volumes following extraction with tional Veterinary Science Laboratory, Ames, Iowa. The virus was propagated RNeasy and the ABI workstation, as described above, a total of 5 and 20 ml and quantitated by plaque assay on Vero cells The RNA samples were Mosquito and bird specimens. A bp fragment nt to containing the analyzed for pathologic signs of viral encephalitis at the Wildlife Pathology Unit C-terminal portion of the C gene and the N-terminal part of the prM gene was of the New York State Department of Environmental Conservation. A ml Valencia, Calif. A total of 5 and 20 ml of RNA of penicillin, and 2. RNA was was tested for each sample. The sample from the standard RT-PCR described above calcium- and magnesium-free or minimal essential medium. A ml reaction mixture contained 13 reac- buffer for extraction with the ABI Prism workstation. A total of 15 ml of Designations in parentheses

are nucleotide number and polarity of the primer-probe. V, viral genomic sense; C, complementary sense. Nucleotide numbering is based on the sequence from Lanciotti et al. After optimiza- tion of the reaction conditions, we found that primer-probe set Downloaded from http: There- fore, it was used for the primary screening of all specimens. Louis encephalitis virus, two common arboviruses in North America, and no signals were obtained data not shown. Two methods were used to quantitate the samples. Sequencea Probe The sequences for the primer-probe sets were designed by R. Lanciotti, CDC 14; Lanciotti, personal communication. We routinely used , 80, 8, 0. Therefore, for data analysis, the threshold line was set at the maximum fluorescence reached by a standard for which the fluorescences of both FAM and TAMRA minimally changed, usually with the 0. Figure 1A shows a plot of the threshold cycles CT; the point at which the fluorescence rises appreciably above the background level versus the log of the titrated amount of virus in PFU with primer-probe set 1. The CT values were The linear dynamic range of the assay is more than 4 orders of magnitude from to 0. We found that although robust and consistent curves were observed with in- Forward primer put virus levels of 0. This was expected since the low level of RNA input less than 0. The unknown samples were analyzed and interpreted by comparing their CT values to those of the standards. The method described above quantitated samples in terms of the number of infectious viral particles. It gave no direct quantitation of the numbers of copies of viral RNA because 39 UTR Targetb not all viral particles are infectious in a virus stock. Therefore, Q solution was added in all subsequent ex- periments. Louis encephalitis virus, and no DNA products were observed, con- firming the specificity of the assay data not shown. To eliminate this possibility, we Downloaded from http: No product was FIG. Similar results were obtained in repeat experiments, and for each experiment, tests with every input concentration were performed in dupli- cate. All RT- of the prM gene, were observed Fig. The intensities of PCR mixtures contained Q solution. Lanes 1 and 9, bp marker. A faint band was visible with input by more than fold. In the absence of Q solution, a standard RT- virus of 0. During the development of the with 0. Lane 1, bp marker. Lanes 2 and 3, mosquito specimens; reagent from the One-Step RT-PCR kit, improved the sensi- lane 4, a bird specimen; lane 1, bp marker. See details in Materials and Methods. Commercial RNA the bp fragment was the result of amplification of viral extraction kits are efficient compared to the traditional phenol- RNA rather than contaminating DNA. To further demon- chloroform extraction method, but they still require manual strate that the product originated from WNV RNA, the am- operation and thus remain a bottleneck for surveillance. The Therefore, the use of an automated RNA extraction system sequencing results confirmed the identity of the amplicons as was essential to increase sample throughput. We tested the WNV. On the basis of the results described above, we chose 8, ABI Prism workstation, a fully automated platform for 0. We spiked a known amount of virus says. Figure 2C presents the results of a representative assay into uninfected bird tissues, homogenized the specimens, and and shows the results for one positive lane 3 and one negative extracted the RNA using either RNeasy Qiagen or the ABI lane 2 mosquito pool and one strongly positive bird sample Prism workstation. The extracted RNA was then quanti- lane 4. For each tissue, we spiked various Nested PCR increases the sensitivity. Although standard amounts of viruses ranging from to PFU. This was expected because the two assays had similar rates by the two extraction methods. No difference in viral sensitivities see above. A more sensitive assay was required RNA recovery was observed for the kidney, heart, brain, and to verify the results for samples with discrepancies. A set of internal prim- factors affected the RNA extraction by either method. As shown in Fig. No DNA product was detected without the agarose gels stained with ethidium bromide. Similar intensities addition of viral RNA lane This was in contrast to the results the results for samples extracted from kidney and heart tissues, obtained by the standard RT-PCR, with a sensitivity of about respectively. Similar results were obtained with brain and 0. These results dem- spleen tissues data not shown. ABI Prism workstation dramatically increases the Up to 96 samples could be processed in approximately 1 h by throughout of RNA extraction, with recovery as efficient as the robotic process, whereas it takes a trained technician ap- that by the RNeasy method. Although real-time RT-PCR sig- proximately 4 h to process the same number of samples by the nificantly increased the throughout of the surveillance assays,

RNeasy method. For confirmatory purposes, the positive samples were then subjected to a secondary assay with primer-probe set 2 or 3 Table 1. Samples that tested positive by both assays were considered confirmed positives. Since primer-probe sets 2 and 3 are less sensitive than set 1, it was expected that some samples with low levels of WNV-specific RNA from the initial screen would be negative by the confir- matory assay. A portion of the specimens that were not confirmed were analyzed further by the nested PCR assay. During surveillance in the year , all the samples were processed by the RNeasy procedure. However, with the introduction of the high-throughput ABI Prism workstation, laboratory ca-

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3: Detection of West Nile virus | Read by QxMD

High-Throughput Detection of West Nile Virus RNA PEI-YONG SHI,1 ELIZABETH B. KAUFFMAN,1 PING REN,1 ANDY FELTON,2 JENNIFER H. TAI,3 ALAN P. DUPUIS II,1 SUSAN A. JONES,1 KIET A. NGO,1 DAVID C. NICHOLAS,1 JOSEPH MAFFEI,1 GREGORY D. EBEL,1 KRISTEN A. BERNARD,1 AND LAURA D. KRAMER1 Wadsworth Center for Laboratories and Research, New York State.*

Brain swab samples from 39 corvids were tested; 27 These findings are similar to previous results for VecTest, which also tested well with House Finches Carpodacus mexicanus , Northern Cardinals Cardinalis cardinalis , and American Kestrels Falco sparverius 4. In the previous New York study, VecTests successfully tested brain, kidney, blood, feather pulp, and cloacal samples from corvids and House Sparrows 4. Brain swab samples may be the preferred antigen source when the oral cavity is compromised. Further testing of alternative swab samples is warranted and may identify a superior antigen source; however, testing internal organs may pose greater risks and may not be applicable in field work and nonlaboratory-based surveillance. In addition, further testing, including immunohistochemical tests, on noncorvids should be conducted to accurately assess these tests and identify the distribution of WNV in the oral cavity and internal tissues. In this study, VecTest produced no false-positive results. Although its specificity was high, RAMP produced 8 false-positive results range The remaining 4 false-positives 3 American Crows and 1 Blue Jay , with scores from VecTest results are easily distinguished when a true WNV-positive reaction occurs, but the reddish-purple line may appear faint or thin in other cases and may be subject to interpretation 4. RAMP quantitative results eliminate subjective interpretation, which helps assure replication but limits confidence in lower RAMP-positive scores. If large numbers of specimens are tested, the cost of the RAMP reader per test is minimal. The RAMP test requires a minimum of 1. Using both tests in a system in which initial testing is conducted with VecTest may also be useful; RAMP could be reserved for high-priority cases in which VecTest results are negative. RT-PCR should still be used to confirm initial viral activity in a new period and area and for research requiring more definitive results. Watt and other animal control officers, wildlife rehabilitators, and the concerned public for coordinating and participating in dead bird reporting and submission. Footnotes Suggested citation for this article: Assays to detect West Nile virus in dead birds. Emerg Infect Dis [serial on the Internet]. Lanciotti RS Molecular amplification assays for the detection of flaviviruses. Comparative sensitivity of the VecTest antigen-capture assay, reverse transcriptase-PCR, and cell culture for detection of West Nile virus in dead birds. Vector Borne Zoonotic Dis. Rapid antigen-capture assay to detect West Nile virus in dead corvids. Evaluation of commercial assays for detecting West Nile virus antigen. Burnaby, British Columbia, Canada:

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4: CiteSeerX â€” DISPATCHES Assays to Detect West Nile Virus in Dead Birds

Field specimens, especially mosquitoes and dead birds, collected as part of surveillance programs, are tested for the presence of viral nucleic acid, viral antigen, or infectious virus. Rapid test protocols have been developed in response to the expansion of WNV in the United States.

Highlight and copy the desired format. Emerging Infectious Diseases, 11 11 , Since the discovery of West Nile virus WNV in New York in , an integral part of monitoring has been testing dead bird tissue by using real-time and standard reverse-transcriptaseâ€"polymerase chain reaction RT-PCR 1 â€" 3. The detection limit for WNV by both methods is as low as 0. Although studies have found that VecTest, with a detection limit in mosquitoes of 5. Disadvantages were occasional atypical results, including false-positives 4. Both tests incorporate immunochromatographic test strips by using labeled antibodies to detect antigen in samples. VecTest uses antibodies bound to gold sol particle labels, while the RAMP test uses antibodies bound to fluorescently labeled latex particles. Development of a visible reddish-purple line in both the test and control zones on the VecTest strip indicates a positive result. The RAMP test strip, enclosed within a cartridge, is inserted into a reader that calculates the ratio between the fluorescence emitted at the test and control zones and displays the results as RAMP units. Values above a background threshold are recorded as positive. Oral swab samples for the RAMP and VecTest were collected with 2 sterile, polyester fiber-tipped plastic applicators held together and moved around the oral cavity and proximal esophagus. One swab sample was twirled in 1. The second swab sample was either twirled in 1. RAMP tests were run the same day on fresh material or later on frozen samples. Before being tested, all frozen samples were thawed at room temperature; swabs not previously mixed in solution and swabs from thawed carcasses were then mixed in RAMP buffer solution. Samples were taken from the brains of a subset of corvid species by swabbing cerebral parenchyma and processing as for oral samples. Differences in test performance were assessed by chi-square analysis. Retests of 6 birds yielded different results from the original tests. Three of these were initially positive and retested negative; the original values were low, which indicated infectivity was focal and undetected on a different sample, and the level was below RAMP and VecTest limits of detection. Three originally negative samples retested positive; 2 were highly positive, which indicated a technical error, and 1 kidney tissue sample was positive, although results of a retest with brain tissue were negative. In this study, oral samples from birds were tested; RAMP sensitivity was The detection thresholds of these tests, coupled with viral titers of specimens, may explain these different results. With the exception of a few species, both tests performed poorly overall on small sample sizes of other noncorvid species. To determine if RAMP results were affected by freezing the sample, samples from 13 corvids 10 positive, 3 negative were retested by using swabs taken from frozen carcasses. Six initially were tested with fresh swabs and 7 with frozen swabs; all retests yielded results similar to initial results. The same results for fresh versus frozen samples were obtained with VecTest 4. VecTest specificity with oral swabs was excellent in correctly identifying all RT-PCRâ€"negative birds, returning no false-positive results Table 2. Brain swab samples from 39 corvids were tested; 27 These findings are similar to previous results for VecTest, which also tested well with House Finches Carpodacus mexicanus , Northern Cardinals Cardinalis cardinalis , and American Kestrels Falco sparverius 4. In the previous New York study, VecTests successfully tested brain, kidney, blood, feather pulp, and cloacal samples from corvids and House Sparrows 4. Brain swab samples may be the preferred antigen source when the oral cavity is compromised. Further testing of alternative swab samples is warranted and may identify a superior antigen source; however, testing internal organs may pose greater risks and may not be applicable in field work and nonlaboratory-based surveillance. In addition, further testing, including immunohistochemical tests, on noncorvids should be conducted to accurately assess these tests and identify the distribution of WNV in the oral cavity and internal tissues. In this study, VecTest produced no false-positive results. Although its specificity was high, RAMP produced 8 false-positive results range The remaining 4 false-positives 3

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American Crows and 1 Blue Jay , with scores from VecTest results are easily distinguished when a true WNV-positive reaction occurs, but the reddish-purple line may appear faint or thin in other cases and may be subject to interpretation 4. RAMP quantitative results eliminate subjective interpretation, which helps assure replication but limits confidence in lower RAMP-positive scores. If large numbers of specimens are tested, the cost of the RAMP reader per test is minimal. The RAMP test requires a minimum of 1. Using both tests in a system in which initial testing is conducted with VecTest may also be useful; RAMP could be reserved for high-priority cases in which VecTest results are negative. RT-PCR should still be used to confirm initial viral activity in a new period and area and for research requiring more definitive results. Watt and other animal control officers, wildlife rehabilitators, and the concerned public for coordinating and participating in dead bird reporting and submission. Molecular amplification assays for the detection of flaviviruses. Virus detection protocols for West Nile virus in vertebrate and mosquito specimens. VecTest as diagnostic and surveillance tool for West Nile virus in dead birds. Vector Borne Zoonotic Dis. Detecting West Nile virus in owls and raptors by an antigen-capture assay. West Nile virus detection in American Crows. Evaluation of commercial assays for detecting West Nile virus antigen. Burnaby, British Columbia, Canada:

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5: High-Throughput Detection of West Nile Virus RNA - www.enganchecubano.com

*High-Throughput Detection of West Nile Virus RNA PEI-YONG SHI, 1 * ELIZABETH B. KAUFFMAN, 1 PING REN, 1 ANDY FELTON, 2 JENNIFER H. TAI, 3 ALAN P. DUPUIS II, 1 SUSAN A. JONES, 1 KIET A. NGO, 1.*

Comment Jan 22, - Elizabeth B. Dupuis II, Kiet A. Bernard, and Laura D. Laboratory testing is the backbone of any surveillance program. Protocols to detect the presence of WNV have been refined since for sensitivity, speed, efficiency, and specificity. This paper presents the protocols currently used by the New York State Department of Health to handle vertebrate and mosquito specimens that have been submitted for WNV testing to the Arbovirus Laboratories of the Wadsworth Center. The virion contains single-stranded, positive-sense RNA approximately 11 kb in length. In the summer and fall of , an outbreak of WNV infection in the northeastern United States was responsible for 62 human cases, including seven deaths. Extensive mortality in crows Corvus spp. Lipkin, Letter, Lancet The virus recurred in and expanded its range to include 12 states in the northeastern United States 3. In , the virus spread dramatically, and by October it had extended its range along the East Coast of the United States from Vermont to Florida and west past the Mississippi River. Its presence was documented in a total of 25 states and the District of Columbia, as well as in Canada 4. The spread was even more dramatic in , with the virus reaching 44 states across the United States and five provinces in Canada and infecting over 3, humans, with at least fatalities 5. Due to the intensive surveillance that was instituted in in New York state, the spread of WNV among mosquitoes, birds, and other vertebrates was monitored effectively. Accumulated data on viral infection among various species of mosquitoes and among birds as well as other vertebrates e. The continuing incursion of WNV into the United States and the increasing numbers of samples submitted to the laboratory for testing necessitate efficient detection procedures for analysis of submitted specimens, which consist of mosquitoes, bird and mammal tissues, sera from human, mammal, and avian sources with suspected infections, and sera from sentinel animals that were placed in locations designed for monitoring of the presence of the virus. Assays were developed for both antibody and virus detection. WNV-specific antibody was detected by standard indirect enzyme-linked immunosorbent assay 6 and plaque reduction neutralization assays. Detection of virus was accomplished through molecular procedures that detected viral nucleic acid, cell culture procedures that detected live virus, and immunologic procedures that detected viral antigen. During the , , and surveillance seasons, our lab used a combination of these methods to evaluate tissues from more than 12, dead birds and 24, mosquito pools collected in New York state. Mosquitoes were collected in the field and sorted by species. Pools of 50 or fewer were placed into 2-ml safe-lock microfuge tubes Eppendorf cat. Dead vertebrates were necropsied at the Wildlife Pathology Unit of the New York State Department of Environmental Conservation, and tissues kidneys, brains, livers, hearts, and spleens were shipped to the Arbovirus Laboratories in individual jars on dry ice. Mosquito pools were homogenized in 0. The remaining mosquito pellet was lysed in 0. If kidney tissue was not available, brain or heart tissue was excised. The amplification reaction was carried out as described previously The sequences of the primer-probe sets were generously provided by Robert Lanciotti 8, Negative controls had water in place of extracted RNA. Tenfold dilutions of WNV standards to 0. Data were analyzed by first identifying the amplification cycle at which fluorescence increased above the threshold CT , which was fixed at 0. A sample was determined to be positive if the CT value was equal to or less than the threshold CT value and the Rn value was two or more times the average Rn value of eight negative wells 8. Results were expressed as CT values or relative numbers of PFU calculated by linear regression from the standard curve. The assay used primer-probe sets 1 env and 2 NS1 , with the probes of sets 1 and 2 labeled with the fluorescent reporter dyes FAM and VIC respectively. The assay was performed exactly as described for single real-time RT-PCR, except that two primer-probe sets were included in the reaction mix, and the concentrations of the primers, 0. The WNV standards were tested in triplicate in both single and multiplex assays to determine sensitivity. Ten replicates of each dilution of the WNV standards were tested to determine

reproducibility. When CPE was observed, infected cells were spotted onto slides, fixed with acetone, and stained by indirect immunofluorescence assay IFA with an immunoglob- J. If immunofluorescence was not observed, new flasks of Vero cells were inoculated with the supernatant harvested from the first passage, infected cells were spotted onto slides, and grouping antibodies against alphaviruses, flaviviruses, rhabdoviruses, and bunyaviruses developed in-house were used to determine etiology. Virus isolates of the family Bunyaviridae were identified to serogroup level by using polyclonal sera against the Bunyamwera and California groups and to species level by using RT-PCR amplification and nucleotide sequencing as described by Huang and others 7. IFA of frozen sections. Indirect IFAs were performed on avian kidney, brain, heart, and spleen. Fluorescence was evaluated by using an Olympus BH-2 microscope equipped with a fluorescein isothiocyanate filter set. Negative controls included negative tissues and a rabies-specific IgM antibody. Ludwig; purchased from Bioreliance, Rockville, Md. Avian samples for which the initial test result was negative were declared to be negative, and no further testing was done. However, negative mosquito samples, other than Culex pipiens complex and Culex restuans, were inoculated onto cell culture see below. Specimens that were positive with two sets of primers were reported as confirmed positive samples. These additional confirmatory tests were used also to further evaluate specimens that represented new species or new locations that had not previously been reported to be associated with WNV. For mammals, all submitted tissues, including brains, kidneys, hearts, livers, and spleens, were screened by real-time RT-PCR. The same algorithm used for mosquito pools and birds was followed; i. In , , and , a total of more than 12, vertebrate specimens and 25, mosquito pools were tested by one or more TaqMan assays at the Wadsworth Center. Cell culture assays for the detection of infectious virus by inoculation of specimens onto Vero cell monolayers and iden- VOL.

6: Publications Authored by Elizabeth Kauffman | PubFacts

West Nile virus (WNV) belongs to the family Flaviviridae. The virion contains single-stranded, positive-sense RNA approximately 11 kb in length. In the summer and fall of , an outbreak of WNV infection in the northeastern United States was responsible for 62 human cases, including seven deaths.

7: Assays to Detect West Nile Virus in Dead Birds - Europe PMC Article - Europe PMC

West Nile Virus Detection in Kidney, Cloacal, and Nasopharyngeal We compared kidney tissue samples and cloacal and In the laboratory, the cloacal and nasopharyn- containing mL BA-1 medium (M salts, % 11, No. 9, September

8: High-Throughput Detection of West Nile Virus RNA | Elizabeth Kauffman and kiet ngo - www.enganchec

Chapter 21 Detection of West Nile Virus Elizabeth B. Kauffman, Mary A. Franke, Susan J. Wong, and Laura D. Kramer Abstract West Nile virus (WNV; Flavivirus, Flaviviridae) is a spherical enveloped virion containing single-stranded.

9: CiteSeerX â€" High-throughput detection of West Nile virus RNA

KAUFFMAN, E. B., BERNARD, K. A., JONES, S. A., MAFFEI, J., NGO, K. and KRAMER, L. D. (), West Nile Virus Laboratory Surveillance Program. Annals of the New York.

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The New Testament, translated into the Cree language 21 SADISTIC BEHAVIOR 427 Asteroid Strikes (Natural Disasters) A Friendship Journey Experiencing archaeology by experiment Brandis and Broun on North Carolina evidence Story boxes, story bags and story telling Architects sketchbooks will jones Best book to learn sanskrit Grails by Bradley H. Sinor Quantification of deep percolation from two flood-irrigated alfalfa field, Roswell Basin, New Mexico Changing Prospects The myth of global chaos Fourier Analysis and Its Applications (Graduate Texts in Mathematics) Techno-rusticity Martyn Wiltshire Flags of the Napoleonic Wars (1 : France and her Allies (Men at Arms, 77) Baseball As America 2004 Wall Calendar Comp Murphys Law 10c Swiss family robinson ebook Leadership paradox Collected papers on Tineina] As in angularjs Trafficking of Intracellular Membranes An Attitudinal Shift and Its Implications 305 Wet foot, dry foot, low foot, high foot Practice tests for Liebert and Spieglers Personality, strategies and issues The perfect pet book Hand-book and directory of Napa, Lake, Sonoma and Mendocino counties Political factors shaping welfare attitudes Test Success Interactive Workbook Statistics with Stata (Updated for Version 7) The Boy Allies on the Firing Line or Twelve Days Battle Along the Marne Community Practice in the Network Society Where the Ox Does Not Plow Fish community results-1990 American material culture and the Texas experience Yo Ho Ho and a Bottle of Milk (Rugrats Files) Essentials of human anatomy physiology 10th edition Pictures in patchwork Colour atlas of the surgery and management of intestinal stomas