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Microbead electrochemiluminescence immunoassay for detection and identification of Venezuelan equine encephalitis virus

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An electrochemiluminescence (ECL) immunoassay, incorporating chemically biotinylated and ruthenylated antibodies down-selected from a panel of monoclonal and polyclonal reagents, was developed to detect and identify Venezuelan equine encephalitis virus (VEEV). The limit of detection (LOD) of the optimized ECL assay was 10^3 pfu/ml VEEV TC-83 virus and 1 ng/ml recombinant (r) VEEV E2 protein. The LOD of the ECL assay was approximately one log unit lower than that of a sandwich enzyme-linked immunosorbent assay (ELISA) incorporating the same immunoreagents. Repetition of ECL assays over time and by different operators demonstrated that the assay was reproducible (coefficient of variation 4.7–18.5% month-to-month; 3.3–8.8% person-to-person). The VEEV ECL assay exhibited no cross-reactivity with two closely related alphaviruses or with 21 heterologous biological agents. A genetically biotinylated recombinant VEEV antibody, MA116SBP, was evaluated for utility for detection of rE2; although functional in the ECL assay, the LOD was two log units higher (100 ng/ml vs 1 ng/ml) using MA116SBP than when chemically biotinylated antibody was used. The ECL assay detected VEEV at the lowest LOD (highest sensitivity) hitherto reported in the published literature and ECL assay results were generated in ~60 min compared to a 6–8 h period required for ELISA. Results have demonstrated a sensitive, rapid, and fully automated ECL immunoassay for detection and identification of VEEV.

1. Introduction

Venezuelan equine encephalitis virus (VEEV), a positive-sense single-stranded RNA alphavirus, is an important mosquito-borne pathogen in humans and equines (Weaver et al., 2004). Epizootic VEEV infections cause debilitating disease with a high fatality rate in equines. In humans, VEEV infection is associated with a potentially life-threatening severe febrile illness and neurological disease appears in ~5% of cases (Griffin, 2001). The incidence of human infection during equine epizootics can reach 30% and mortality associated with encephalitis in children is as high as 35%. The outbreaks in Venezuela and Colombia in 1995 affected around 75,000 people and more than 300 fatal encephalitis cases occurred (Rivas et al., 1997). VEEV is environmentally stable and highly infectious by aerosol inhalation, making it a potential biological weapon threat. As such, it is crucial that rapid and sensitive immunoassays be developed for detection of VEEV in environmental and clinical samples. A number of immunoassay techniques utilizing polyclonal or monoclonal antibodies (Wang et al., 2005) or recombinant antibodies (Duggan et al., 2001; Hu et al., 2002, 2004; Kirsch et al., 2008) has been described for the detection of VEEV, including enzyme-linked immunosorbent assay (ELISA), radioimmunosorbent assay (RIA), light addressable potentiometric (LAP) assay, and dissociation-enhanced lanthanide fluorescent immunoassay (DELFIA) (Roehrig et al., 1982; Smith et al., 2001; Hu et al., 2002, 2004). Typically, these assays require multiple operational steps and long incubation periods; acquisition and analysis of assay results can take many hours.

Electrochemiluminescence (ECL) immunoassay is a magnetic bead-based technology for conducting immunoassays with improved assay performance (Deaver, 1995; Yang et al., 1994). There are three components in an ECL immunoassay: (i) a biotinylated capture antibody (Cab), pre-bound to streptavidin-coated magnetic beads, (ii) a detector antibody (Dab), labeled with ruthenium-trisbipyridal, for the emission of light when electrochemically stimulated, and (iii) an analyte, which reacts with the capture and detector antibodies resulting in an antigen–antibody sandwich. In ECL reactions, a precursor molecule (tripropylamine) is activated on an electrode surface resulting in an electron transfer reaction. This transfer initiates the excitation of ruthenium-trisbipyridal which ultimately results in the emission of a photon at 620 nm.

Assay techniques utilizing ECL technology have a number of advantages over conventional assay methods e.g., ELISA and RIA:

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limits of detection (LOD) in ECL assays are lower (higher sensitivity), due to high luminescent signal to noise ratios (Gatto-Menking et al., 1995; Yu et al., 2000; Garber and O'Brien, 2008; Yoshimura et al., 2008; Rossi et al., 2008; Kuhle et al., 2010). The dynamic range for analyte detection extends over a wide range (five orders of magnitude) (Yang et al., 1994; Kijek et al., 2000; Yu et al., 2000). The time and labour required to complete ECL assays are reduced compared with conventional immunoassays. The ECL assay is a non-separation technique, thus does not require plate coating, washing, or aspiration steps. Sample reading is rapid; the ECL instrumentation used in the present study requires approximately 1 min per sample to read. Labeled ECL reagents are exceptionally stable and ECL assays are robust and tolerant of analyte detection in the presence of a variety of sample matrices. These features make the ECL detection system an attractive alternative to conventional immunoassay techniques. In addition, the instrumentation used in this study is a military hardened version, making it especially useful for military field use.

This study describes the development of an ECL assay for VEEV. A panel of VEEV antibodies was screened for optimal performance using VEEV strain TC-83 whole virus and VEEV recombinant (r) envelope E2 protein as target antigens. ECL assays were optimized for antibody concentration and assay LOD were determined in comparison to ELISA incorporating the same antibody reagents. ECL assays were assessed for reproducibility and precision over time and when conducted by different operators. Assay specificity for VEEV was evaluated by screening against closely related alphaviruses and 21 heterologous bacteria, virus, or toxin agents. A genetically biotinylated recombinant antibody to VEEV was compared to a chemically biotinylated antibody for utility for detection of VEEV rE2.

2. Materials and methods

2.1. Instrumentation

ECL measurements were performed using a M-SERIES® M1MR analyzer (BioVeris Corp., Gaithersburg, MD).

2.2. Antigens

2.2.1. Homologous antigen

Live VEE TC-83 virus, purified as described previously (Hu et al., 2004), was kindly provided by Dr. J. Wu, DRDC Suffield. Recombinant E2 protein was expressed in *Escherichia coli* and purified at DRDC Suffield (W.-G. Hu et al., unpublished data).

2.2.2. Heterologous antigens

Gamma-irradiated Western equine encephalitis virus (WEEV) and Eastern equine encephalitis virus (EEEV) were purchased from the US Critical Reagents Program (CRP) (Aberdeen Proving Ground, MD). Yellow fever virus, attenuated vaccine (strain 17D), was purchased from Aventis Pasteur Inc. (Toronto, ON). Dengue and *Klebsiella pneumoniae* were purchased from American Type Culture Collection (Manassas, VA). Inactivated (^{60}Co irradiated) *Brucella melitensis* (suis), *Bacillus anthracis* (vollum), *Bacillus globigii* (spores), *Francisella tularensis*, *Yersinia pseudotuberculosis*, *Yersinia pests* (JAVA 9), *E. coli*, *Bacillus cereus*, Vaccinia virus (Lister), *Bacillus thuringiensis*, *Aspergillus niger*, *Erwinia herbicola*, *Pseudomonas aeruginosa*, *Coxiella burnetii*, and MS2 were all acquired from US Army Dugway Proving Ground (DPG) (Dugway, UT). SEB toxin was purchased from Toxin Technology (Sarasota, FL), botulinum toxoid A was purchased from WAKO Chemicals Inc. (Richmond, VA), and ricin A chain was purchased from Sigma–Aldrich Canada Ltd. (Oakville, ON).

2.3. Monoclonal and polyclonal antibodies

Hybridomas 1A4A1 and 1A3A9 were kindly provided by Dr. J.T. Roehrig (Mathews and Roehrig, 1982; Roehrig et al., 1982; Roehrig and Mathews, 1985). The hybridomas were grown in BD Cell™ MAb Basal Medium (BD-Biosciences, Mississauga, ON) supplemented with 10% FETALCLONE® fetal calf serum (HyClone Laboratories, Logan, UT), 2 mM L-glutamine (Invitrogen, Burlington, ON), and 1% HAT Media Supplement Hybri-Max® (Sigma–Aldrich, Canada). The hybridomas were weaned from FBS medium and transferred to a CELLine™ 1000 flask (BD-Biosciences) and incubated at 37 °C under 5% CO_2 for 2 weeks. Supernatant was harvested from the CELLine™ 1000 flask and the monoclonal antibody (mAb) was then purified using a Melon™ Gel Monoclonal IgG Purification Kit (Pierce, Ottawa, ON) according to the manufacturer's instructions.

Rabbit and goat anti-VEEV polyclonal antibodies (pAb) were developed previously and purified for IgG under a DRDC Suffield contract by SciLab Consulting Inc. (Redcliff, AB). Both the rabbit and the goat pAb had been purified on a Bio-Gel® Protein G column (Bio-Rad Laboratories, Mississauga, ON) by High-Performance Liquid Chromatography (Spectral Physics, San Jose, CA).

2.4. Genetically biotinylated recombinant antibody

A genetically biotinylated recombinant VEEV single-chain variable fragment antibody, MA116SBP, was previously generated at DRDC Suffield (Alvi et al., 1999, 2002, 2003; Hu et al., 2002).

2.5. ECL assay reagents

The following ECL assay reagents and buffers were purchased from BioVeris Corp.: biotin-LC-Sulfo-NHS ester, ruthenium (II) tris-bipyridine-NHS ester, streptavidin-coated Dynabeads® M-280, M-SERIES® Positive Calibrator, M-SERIES® Negative Calibrator, StabilCoat® Immunoassay Stabilizer, BV-CLEAN™ Plus solution, BV-GLO™ Plus solution, BV-DILUENT™ solution, BV-STORE™ solution, and BV-SANITIZE™ solution.

2.6. Matrix powders and soils

Flour (white, enriched, all purpose) (Safeway brand), cornstarch (Safeway brand), baking powder (Safeway brand), baking soda (Safeway brand), laundry detergent (Tide Original, Procter and Gamble), coffee creamer (Coffee-Mate®, Carnation, Nestlé), skim milk, and powdered sugar were all purchased from Safeway Canada Ltd. (Medicine Hat, AB). Talcum powder, powdered cleanser, and spackling powder were purchased through Foreign Military Sales from the US Critical Reagents Program. The four soil samples including sand, sand loam, loamy sand, and clay loam were from the DRDC Suffield Experimental Proving Ground and had been previously characterized by the Alberta Environmental Centre (Vegreville, AB).

2.7. Preparation of labeled antibodies

Prior to labeling, all antibodies were desalted using NAP-5 columns (Amersham Biosciences, Baie d'Urfé, QC) according to the manufacturer's instructions. In separate labeling reactions, the four antibodies (1A4A1 mAb, 1A3A9 mAb, rabbit pAb, and goat pAb) were each biotinylated with biotin-LC-Sulfo-NHS ester at a molar ratio of 10:1 (biotin:Ab) and ruthenylated with ruthenium (II) tris-bipyridine-NHS ester at 7.5:1 (Ru:Ab) by labeling procedures recommended by BioVeris. In brief, the antibodies and respective labeling solutions were incubated separately and simultaneously using an end-over-end shaker with gentle rotation for 60 min at RT. The labeling reactions were quenched by the addition of 20 μl