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A handheld NASBA analyzer for the field detection and quantification of *Karenia brevis*

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Abstract

Blooms of *Karenia brevis*, the red tide forming dinoflagellate in the Gulf of Mexico, cause a myriad of ecological and economic problems for coastal communities, including massive fish and mammal mortalities, and damage to tourism and fisheries/shellfish harvesting industries. There is a need for accurate detection and prediction of *K. brevis* blooms, including rapid and inexpensive monitoring of both water and shellfish meats to ensure the safety of shellfish harvested for human consumption. To address this issue, we have developed a protocol for easy field extraction of cellular RNA from water samples and coupled it with a handheld nucleic acid sequence-based amplification (NASBA) sensor that amplifies and detects target mRNA specific to the *rbcL* gene of *K. brevis*. This extraction protocol is a modified version of the Qiagen RNeasy Mini Kit spin protocol and requires no specialized equipment or training. Once extracted, the RNA is amplified and detected by NASBA in an in-house designed and produced handheld sensor that provides a real-time fluorescence plotting of the amplification. Both the field RNA extraction protocol and the handheld NASBA analyzer compared favorably to laboratory-based technologies. In duplicate reactions, the amplification curves generated with the handheld detector closely mirrored the curves generated with the bench top Nuclisens EasyQ NASBA analyzer and there was no difference in the sensitivity obtained using the handheld device versus the bench top models. This extraction protocol and detection sensor will be a valuable tool for rapidly monitoring *K. brevis* in field environments.

Keywords: Field detection; *Karenia brevis*; NASBA; Quantification

1. Introduction

Blooms of the Florida red tide dinoflagellate *Karenia brevis* (formerly *Gymnodinium breve*) have plagued the Gulf coasts since the Spanish conquests and continue to inflict damage to marine life, coastal resource users, fisheries, tourism, and human health. Red tides, a near yearly event along the Florida Gulf coast, are responsible for massive fish kills and marine mammal mortalities (Landsberg, 2002; Landsberg and Steidinger, 1998). Impacts on human health due to red tides include eye irritation and respiratory distress from inhalation of the aerosolized toxin, as well as paralytic shellfish poisoning via ingestion of contaminated shellfish. Economic costs due to red tides in Florida are difficult to estimate, but likely exceed US$ 20 million per year from losses in tourism alone (Anderson et al., 2000). The Florida shellfishing industry, which employs over 2500 workers and whose annual value is greater than US$ 20 million

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per year, is also negatively impacted by red tides when blooms result in the closure of shellfish harvesting beds (http://www.floridaaquaculture.com/SEAS/SEAS_intro.htm).

The rapid detection of harmful microorganisms in seawater is vital for monitoring the safety and health of coastal waters. Maintenance of healthy waters for shellfish harvesting, recreational water contact usage, and detection of harmful algal blooms all require rapid, sensitive, and specific assays for noxious microbial detection. Current monitoring of *K. brevis* relies on manual counting of cells, which is time consuming and requires trained personnel. Additionally, samples must be brought back to the laboratory prior to analysis resulting in a lag between sample collection and generation of information on blooms. Rapid, quantitative methods are needed to detect blooms in early stages, to provide information to resource managers for monitoring shellfish beds, and to understand the ecology of this organism in the field.

The overall goal of this research is to develop portable sensor technology for detection and enumeration of *K. brevis* in coastal water using a previously developed nucleic acid sequence-based amplification (NASBA) assay for the molecular detection of *K. brevis* (Casper et al., 2004; Patterson et al., 2005). NASBA is an isothermal method for the amplification of RNA (Compton, 1991). Coupled with molecular beacon technology, NASBA becomes a quantitative, target-specific detection method (Leone et al., 1998). We have developed a simple field RNA extraction protocol that does not require specialized laboratory equipment or training, to be used in conjunction with an in-house designed (Center for Ocean Technology, St. Petersburg, FL) handheld NASBA incubator and fluorescence analyzer for in-the-field, quantitative detection of *K. brevis*. Together, these technologies provide a portable assay that detects *K. brevis* in as little as 20 min. Furthermore, this device is an inexpensive alternative to existing laboratory instrumentation.

2. Materials and methods

2.1. K. brevis

K. brevis cultures (Piney Island and Charlotte Harbor strains) were obtained from The Fish and Wildlife Research Institute (FWRI) in St. Petersburg, FL. Cultures were incubated at 24 °C on a 12 h light:12 h dark cycle at 26 μmol photon^{-2}. Prior to RNA extraction, cell concentration was determined by filtering 1 ml of culture onto 0.22 μm pore size black polycarbonate membranes (Millipore Corp., Billerica, MA) and cells were counted under epifluorescence microscopy (Olympus BH-2 microscope) using blue excitation (Vernet et al., 1990). The appropriate volume of culture was then used for RNA extraction to generate standard curves for quantitative analysis.

2.2. RNA extraction

K. brevis RNA was extracted using either an RNeasy Mini Spin Kit (Qiagen Corp., Madison, WI) or the field RNA extraction protocol. The RNA field extraction protocol was developed by modifying the RNeasy Mini Spin Kit protocol and RNA extracted using this method was obtained as follows: a custom manufactured adapter (Center for Ocean Technology) was designed to connect the RNeasy spin column to a 60 ml syringe. Using the attached syringe, a 20–30 ml water sample was pushed through the spin column. The syringe and adapter were removed, and the column placed into a collection tube. RLT lysis buffer plus β-mercapto ethanol (10 μl β-me/ml RLT) was mixed with 100% ethanol in a ratio of 7:5. Seven hundred microliter of the RLT/EtOH mixture was applied to the RNeasy spin column and incubated at room temperature for 10 min. After the 10 min incubation, the spin column was removed from the collection tube and one end of a Value Plastics (Fort Collins, CO) female luer lug style coupler (part# FTLC-6) was attached to the bottom of the column. A 10 ml syringe was attached to the other end of the coupler, and the RLT/EtOH mixture was slowly drawn through the column by pulling the plunger. Leaving the syringe attached to the column, 700 μl of Qiagen's RWI buffer was added to the column and slowly drawn though with the syringe. Two washes of 500 μl RPE were applied to the column and pulled through as described above. After the second RPE wash, the syringe was removed from the column and capped with a Value Plastics female luer thread style cap (part# FTLLP-6) and stored for disposal. A clean 60 ml syringe, with the plunger pulled back, was attached directly to the spin column. The bottom of the spin column was allowed to rest on absorbent paper. While holding the syringe to the column, the plunger was depressed quickly to push air through the column and remove residual wash buffer. This was repeated at least five times or until no more liquid was expelled onto the paper. The syringe was removed and the spin column was placed in a collection tube. Fifty microliters of RNase free water was applied to the spin column. Using the same 60 ml syringe that was used to dry the column, the RNA was eluted from the column by pushing air through in the manner described above.