Performance evaluation of a Bayer Healthcare Diagnostics research-based SARS coronavirus assay

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Introduction

Severe acute respiratory syndrome (SARS) is an emerging infectious disease caused by a highly contagious coronavirus (SARS-CoV) that is transmitted through direct or indirect contact with secretions from mucous membranes [1, 2]. The disease first appeared in southern China in late 2002, and rapidly spread to 31 countries within the first 6 months of 2003 [3–5]. At the end of this epidemic, the World Health Organization (WHO) estimated the number of probable SARS cases at 8,098 with 774 deaths [6]. Since the initial epidemic was declared over by the WHO in July 2003, there have been several new laboratory confirmed cases of SARS in late 2003 and 2004 resulting from accidental research laboratory exposure (in Singapore, Taiwan and China) and animal or environmental exposure (in China) [7–10].

A recent Centers for Disease Control and Prevention (CDC) guideline for clinical specimen collection, diagnostic testing and an interpretation algorithm for SARS-CoV infection has been developed [11]. According to these guidelines, respiratory tract, blood and stool are the preferred specimens for serological and molecular diagnostic testing through the clinical course of the disease. Several enzyme immunoassays and immunofluorescent assays for serological diagnosis and reverse-transcriptase PCR (RT-PCR) assays for detection of SARS-CoV RNA in clinical specimens have been described [12–15].

Bayer Healthcare Diagnostics developed a research based RT-PCR assay for detection and quantification of SARS-CoV in clinical specimens during the 2002–2003 outbreak of SARS. (This assay is for research use only, not for use in diagnostic procedures.) This article describes this assay, together with the assay’s validation and performance and compares it with the
RealArt™ HPA-Coronavirus LC RT PCR Kit (Artus GmbH, Hamburg, Germany) for detection of SARS-CoV RNA in clinical specimens.

SARS coronavirus detection assays

In an astounding research accomplishment, the full-length genome sequence of the SARS-CoV was available within weeks after the identification of the pathogen and the initial global WHO alert [16, 17]. The availability of the nucleotide sequence allowed for the development of specific molecular diagnostic assays to detect SARS-CoV RNA in clinical specimens.

Bayer Healthcare Diagnostics developed a one-step, real-time quantitative RT-PCR assay for SARS-CoV RNA quantification. Full genomic sequences of SARS-CoV were used to design homologous forward and reverse primers and fluorescent labeled TaqMan probe targeting a 67-base pair nucleocapsid genomic region. Primer and probe design included a BLAST search with other human coronaviruses and human genomic DNA to exclude sequence cross reactivity [18]. A second heterologous amplification system including forward and reverse primers, fluorescent labeled TaqMan probe and target was included as an internal control for the assay process including sample preparation and PCR amplification.

One-step amplification reactions were performed using the Qiagen® OneStep RT-PCR kit (Qiagen, Valencia, CA, USA) in a reaction volume of 25 µl containing 5 µl of target (extracted patient tissue RNA and heterologous internal control RNA). Reactions were first incubated at 55°C for 30 min to complete the reverse transcriptase step followed by incubation at 95°C for 15 min to inactivate the RT enzyme. Reactions were then thermocycled with the following parameters: denaturation at 95°C for 30 sec followed by 40 cycles of 60°C for 60 sec and 72°C for 30 sec. The Stratagene MX3000PTM Real-Time PCR System (Stratagene, La Jolla, CA, USA) was used to analyze the emitted fluorescence during amplification. Positive and negative controls, containing standardized SARS viral culture RNA extract from cell culture supernatants of VeroE6 cells (National Center for Infectious Diseases, CDC, Atlanta, GA, USA) and nuclease-free RNA diluent, respectively, were included in each run. External standards for SARS-CoV RNA quantification were prepared with serial dilutions of quantified SARS-CoV RNA culture extract, with concentrations ranging from 10 to 10^6 copies/5 µl reaction. The quantification standards were value assigned in comparison with three lots of purified RNA transcripts generated from the nucleocapsid region cloned into a plasmid (pCR-N9). The plasmid was provided through a material transfer agreement with the CDC. The RNA transcripts were prepared from the T7 promoter using the MEGAscript™ In Vitro Transcription Kit (Ambion, Austin, TX, USA). The concentration of the transcripts was determined by measuring the absorbance at 260 and