Use of sodC versus ctrA for real-time polymerase chain reaction-based detection of Neisseria meningitidis in sterile body fluids

Fábio Takenori Higa1*, Lucila Okuyama Fukasawa1, Maria Gisele Gonçalves1, Maristela Marques Salgado1, Ana Paula Silva de Lemos2, Lee H Harrison1, Priscilla Lima de Oliveira1, Carla Naufal da Silva1, Claudio Tavares Sacchi1

1Centro de Imunologia 2Centro de Bacteriologia, Instituto Adolfo Lutz, São Paulo, SP, Brasil 3Infectious Diseases Epidemiology Research Unit, Graduate School of Public Health, School of Medicine, University of Pittsburgh, Pittsburgh, PA, USA

We evaluated the use of a newly described sodC-based real-time-polymerase chain reaction (RT-PCR) assay for detecting Neisseria meningitidis in normally sterile sites, such as cerebrospinal fluid and serum. The sodC-based RT-PCR assay has an advantage over ctrA for detecting nongroupable N. meningitidis isolates, which are commonly present in asymptomatic pharyngeal carriage. However, in our study, sodC-based RT-PCR was 7.5% less sensitive than ctrA. Given the public health impact of possible false-negative results due to the use of the sodC target gene alone, sodC-based RT-PCR may be used with caution.

Key words: sodC-based RT-PCR - N. meningitidis diagnostics - meningitis

Meningococcal disease (MD) is a serious and often fatal infection. In Brazil, approximately 3,200 new cases of sporadic and outbreak-associated MD are reported each year, making this disease an important public health concern. Our laboratory at Adolfo Lutz Institute (IAL) uses a multiplex real-time-polymerase chain reaction (RT-PCR) assay developed and performed at IAL (Sacchi et al. 2011). The assay is composed of three sets of primers and three probes targeting the ctrA gene of Neisseria meningitidis (Mothershed et al. 2004), the lytA gene of Streptococcus pneumoniae (Carvalho et al. 2007) and the bexA gene of Haemophilus influenzae (Corless et al. 2001). ctrA is a frequently targeted gene used to detect N. meningitidis using RT-PCR (Corless et al. 2001, Mothershed et al. 2004). However, the capsule locus, including ctrA, is subject to rearrangement (Swartley et al. 1997, Clauds et al. 2002, Dolan-Livengood et al. 2003, Sadler et al. 2003) and 16% or more of carried meningococci have been shown to lack ctrA altogether (Clauds et al. 2002, Dolan-Livengood et al. 2003).

It has been suggested that invasive meningococci can undergo similar rearrangements of the capsule region. For these reasons, a new target based on the sodC gene in meningitidis RT-PCR has been proposed as an alternative for clinical specimens, such as cerebrospinal fluid (CSF) and serum (Thomas et al. 2011, WHO 2011) to detect N. meningitidis and a capsule gene-based RT-PCR for meningococcal genogrouping (Mothershed et al. 2004). DNA was extracted using a MagNA Pure LC and the DNA Isolation Kit III according to the manufacturer's instructions (Roche Diagnostics GmbH, Mannheim, Germany). All extracted DNA was stored at -20°C. The assays were performed in a 25 μL reaction volume using TaqMan Universal Master Mix (Applied Biosystems, Foster City, CA) with 5 μL of extracted DNA. Forward primer, reverse primer and the probe for each gene target were used at previously described concentrations.

All reactions were run in duplicate using an Applied Biosystems 7500 Real-Time PCR System (Applied Biosystems Foster City, CA, USA) with the following cycling parameters: 50°C for 2 min, 95°C for 10 min and 45 cycles of 95°C for 15 s and 60°C for 1 min. Extension at 55°C for 1 min was used for RT-PCR genogrouping for serogroups B, W135 and Y. Positive and negative controls were included in each run. A review of the literature showed that no standards exist for determining what criteria indicate a positive result. Some researchers established a “cut-off” cycle threshold (Ct) value for a particular assay, while others considered any amplification signal, regardless of the Ct threshold, a positive result. Some laboratories have used a single amplification signal, regardless of the Ct
value, indicative of a positive result. However, the rationale for determining such parameters was not always clear. In this study, a positive result was defined as a Ct ≤ 38 and a negative result as a Ct value of zero or ≥ 39. All inconclusive results and inconsistent replicates were repeated. The lower limit of detection (LLD) for sodC and ctrA RT-PCR assays at a Ct of 38 was 200 fg. The efficiency of both reactions was similar: 92.15% for ctrA and 90.42% for sodC.

The results were classified into four groups: Group A, positive for both ctrA and sodC; Group B, positive for ctrA and negative for sodC; Group C, negative for ctrA and positive for sodC, and Group D, negative for both ctrA and sodC (Table). All samples that were positive for sodC were also positive for ctrA (Group A), but 24 samples that were positive for ctrA were negative for sodC (Group B). The Ct values of ctrA and sodC for each sample in Group A were mostly similar, varying from 23-38. To compare our results with the original paper that described the use of sodC for detecting N. meningitidis (Thomas et al. 2011), we also used a second Ct value of ≤ 35 to define a positive result. We were only able to obtain culture results for 75 samples (51 from Group A and 24 from Group B).

**Group A** - Among the 295 Group A samples, 87.1% (257/295) had a Ct ≤ 35 for both sodC and ctrA, 7.8% (23/295) had a ctrA Ct ≤ 35 and a sodC Ct ≥ 36 (10 that were culture-confirmed) and 5.1% (15/295) had a Ct ≥ 36 (8 that were culture-confirmed) for both sodC and ctrA. Therefore, 38 Group A samples (18 culture-confirmed) were considered negative using a sodC Ct ≤ 35 to define a positive result.

**Group B** - Among the 24 Group B samples, seven had ctrA Ct values ≤ 35: Ct = 29, 31, 32 and 33 (1 of each) and Ct = 35 (n = 3). Additionally, 42% (10/24) were isolated from the CSF and 58% (14/24) were isolated from the serum. No samples had less than the ideal volume. Three of 24 samples that were culture confirmed had ctrA Ct values of 35, 36 and 37 and the whole-cell suspensions made from the three isolates were positive for ctrA and sodC.

We were able to determine the genogroup of all 319 ctrA-positive samples (Groups A and B): 17.2% were genogroup B (n = 55), 76.5% were genogroup C (n = 244), 3.8% were genogroup W (n = 12) and 2.5% were genogroup Y (n = 8).

A sodC-based RT-PCR assay has an advantage over ctrA in detecting nongroupable N. meningitidis isolates, which are common in asymptomatic pharyngeal carriage. However, in our study, sodC-based RT-PCR was 7.5% less sensitive than ctrA in normally sterile body fluids. Our data are discordant from those previously reported by Thomas et al. in 2011. According to our data, there was no suggestion that these differences may be due to sample type or volume or differences in the LLD. Unfortunately, we could not determine the reasons for these differences. However, we analysed a much larger and more diverse collection representing 220 health care units distributed throughout the state of São Paulo.

Given the public health impact of possible false-negative results due to the use of the sodC target gene alone, using sodC-based RT-PCR to detect N. meningitidis should be performed with caution.

### REFERENCES


---

**Table**

<table>
<thead>
<tr>
<th>Group</th>
<th>ctrA</th>
<th>sodC</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Positive</td>
<td>Positive</td>
<td>295 (19.2)</td>
</tr>
<tr>
<td>B</td>
<td>Positive</td>
<td>Negative</td>
<td>24 (1.6)</td>
</tr>
<tr>
<td>C</td>
<td>Negative</td>
<td>Positive</td>
<td>0 (0)</td>
</tr>
<tr>
<td>D</td>
<td>Negative</td>
<td>Negative</td>
<td>1,219 (79.2)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td>1,538 (100)</td>
</tr>
</tbody>
</table>