

Respiratory illness caused by *Corynebacterium diphtheriae* and *C. ulcerans*, and use of diphtheria anti-toxin in the United States, 1996-2018: Supplementary Material

METHODS

Conventional PCR Assay

The conventional PCR described by Nakao and Popovic [29] was used to test and report results of specimens collected between 1996-2000. This PCR used two sets of primers in separate reactions targeting the A and B subunits of the diphtheria toxin gene (*tox*). Primers Tox 1 and Tox 2 amplified a 248 base pair region of *tox* subunit A and primers Dipht 6F and Dipht 6R amplified a 297 base pair region of *tox* subunit B. The products of the PCR reactions were electrophoresed in an agarose gel and, after staining with ethidium bromide, DNA bands were read using UV light.

Real-time PCR Assay

The real-time PCR described by Mothershed et al [30] was used to test and report results for specimens collected between 2001-2017. This PCR used two sets of primers, each with a corresponding probe tagged with a fluorescent dye, in separate reactions targeting the A and B subunits of *tox*. The subunit A primers and probe amplified a 117 base pair region of *tox* subunit A and the subunit B primers and probe amplified a 128 base pair region of *tox* subunit B. Reactions were carried out in 96-well plates and read in real-time by the thermocycler.

Coryne Triplex Real-time PCR Assay

Specimens from 2018 to present have been tested using a novel real-time PCR method developed by Williams et al [31]. This assay includes the addition of a targets specific for *C. diphtheriae* and *C. ulcerans/C. pseudotuberculosis*, while using a single target for *tox* instead of separate A and B subunit targets. The three sets of primers and probes are combined into a single triplex reaction.

SUPPLEMENTARY FIGURE LEGEND

Supplementary Figure: Reported diphtheria cases in the United States, 1996-2018, by type of laboratory test result. The checkered bar represents cases for which no laboratory testing was completed. The wavy bar represents cases for which culture testing was negative for *C. diphtheriae* and PCR testing for *tox* was negative. The dotted bar represents cases for which culture testing was negative for *C. diphtheriae* and PCR testing for *tox* was positive. The blank bar represents cases for which culture testing was positive for *C. diphtheriae* but no testing for toxigenicity (Elek test) or PCR was available. The horizontally lined bar represents cases for which culture testing was positive for *C. diphtheriae* and toxigenicity testing (Elek test) was negative. The black bar represents cases for which culture was positive for *C. diphtheriae* and toxigenicity testing (Elek test) was positive.