DEPARTMENT OF HEALTH & HUMAN SERVICES Public Health Service

Centers for Disease Control
and Prevention (CDC)
Atlanta, GA 30341-3724

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Dear Dr. Casey,

This letter is in response to the recent request for data and information on technologies used to detect and measure botulinum neurotoxin (Fed Reg. 83(114): pages 27622-27623, June 13, 2018). The Centers for Disease Control and Prevention (CDC) is the premier public health institute in the United States and considers the accurate diagnosis of human botulism to be an important public health goal. To that end, we have developed a method to detect botulinum neurotoxin (BoNT) in clinical and selected food matrices.

The method is called the Endopep-MS method and detects the neurotoxin based on its enzymatic activity. The Endopep-MS method involves concentration and isolation of BoNT from a complex matrix using carefully selected monoclonal antibodies, followed by incubation with a substrate that is enzymatically cleaved by BoNT. The resulting substrate product is analyzed by mass spectrometry to determine the presence or absence of the BoNT serotype-specific peptide cleavage products indicating the presence of BoNT in the original test sample. Because each serotype produces peptide cleavage products of different mass, the method easily differentiates between all seven confirmed serotypes.

The method was first published in 2005, describing the ability to detect the neurotoxin and differentiate all seven serotypes in buffer [1,2]. Next, the method was adapted to detect BoNT in milk [3], serum [4], stool [4], culture supernatants [5], drinking water [6], and foods [7]. The method has been modified to increase sensitivity and specificity through substrate modification [8-13]. The method can be quantitative [14,15], but is typically run in the qualitative mode to confirm a diagnosis of botulism.

Endopep-MS has undergone extensive validation both within CDC as well as in outside laboratories [16,17]. Within CDC, the validation has consisted of a variety of tests, beginning with a spiked limit of detection study in buffer, serum, stool, and culture supernatants. The limit of detection varies according to matrix and serotype. Diagnostic sensitivity (a positive result from a true positive sample) is in the range of 0.1 mLD50 to 1 mLD50 at the 95% or above confidence interval, or below the limit of detection of the mouse bioassay. In addition to being more sensitive than the mouse bioassay, the assay has smaller sample volume requirements than the mouse bioassay. Endopep-MS has been shown to detect more than just the commercially-available subtypes [18-21], a critical factor for public health testing and part of diagnostic sensitivity that many in vitro assays ignore. To date, the Endopep-MS method has been used to successfully test all subtypes which we have been able to collect, including all of the eight BoNT/A subtypes, seven of the eight BoNT/B subtypes, six of the twelve BoNT/E subtypes, all seven of the BoNT/F subtypes, two BoNT/C subtypes, two BoNT/D subtypes, mosaics of these toxins, and BoNT/G. Endopep-MS has been used in the discovery of novel subtypes [22-28]; the method was used to discover the first new BoNT cleavage site in over 20 years [22].

Diagnostic specificity (a negative result from a true negative sample) was also part of our validation work; diagnostic specificity was found to be 100% as determined using the following samples: negative buffer (minimum of 20 samples), negative serum (minimum of 50 separate specimens), stool extract (minimum of

50 separate specimens), culture supernatants (minimum of 20 samples); and near neighbors of BoNT including tetanus toxin and culture supernatants from related non-BoNT producing *C. botulinum*.

Following validation of the Endopep-MS method, the method was certified as a CLIA diagnostic for human botulism in our laboratory and has been in use for almost a decade, testing clinical specimens (serum, stool, foods, and culture supernatants) in parallel with the mouse bioassay, the standard for BoNT detection, when sample volumes permit the use of Endopep-MS. To date, over 400 samples have been analyzed with both methods with a concordance rate of approximately 99%; a remarkable feat given the increased sensitivity of the Endopep-MS method compared to the mouse bioassay. The method has been applied successfully to a number of public health investigations of botulism [29,30] and was reported as the suggested strategy for detection of BoNT by mass spectrometry [31].

We are currently seeking FDA clearance for the Endopep-MS assay as a 510(k) De Novo In Vitro Diagnostic for human botulism. We have already submitted 2 pre-submission packets of information from our analytical study, our multi-center evaluation, and our clinical study. We are currently engaged in a multi-center validation of the assay involving state public health laboratories within our laboratory response network (LRN). To date, we have trained a number of laboratories on the Endopep-MS method, including 9 laboratories within the LRN, two FDA laboratories, and laboratories in France, Belgium, Germany, Sweden, Czech Republic, and Canada (both PHAC and Health Canada).

In short, the Endopep-MS method is a viable alternative to the mouse bioassay to diagnose human botulism. We are happy to provide additional information upon request.

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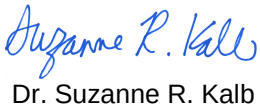
Sincerely,



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