Single-molecule DNA mapping for pathogen detection

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Abstract: Detection and identification of *Clostridium botulinum* is of particular concern due to the extreme pathogenicity of its toxin. Herein, we review the potential applicability of two novel technologies for detection of *Clostridium botulinum* and botulinum toxin. Genome sequence scanning (GSS) provides strain-specific bacterial identification from complex bacterial mixtures by detecting the distribution of fluorescent sequence-specific probes along long linearised DNA fragments. Digital DNA (DD) utilises long fluorescent labelled DNA molecules as binary labelled tags to facilitate multiplex antigen detection. Both technologies share automated sample preparation and detection instrumentation. GSS and DD have particular applicability for botulinum detection and identification, but have broad-reaching applicability to food safety testing and clinical diagnostics in general.

Keywords: botulinum; bacterial detection; strain-typing; toxin identification; digital DNA; DD; genome sequence scanning; GSS; single-molecule; multiplex assay.

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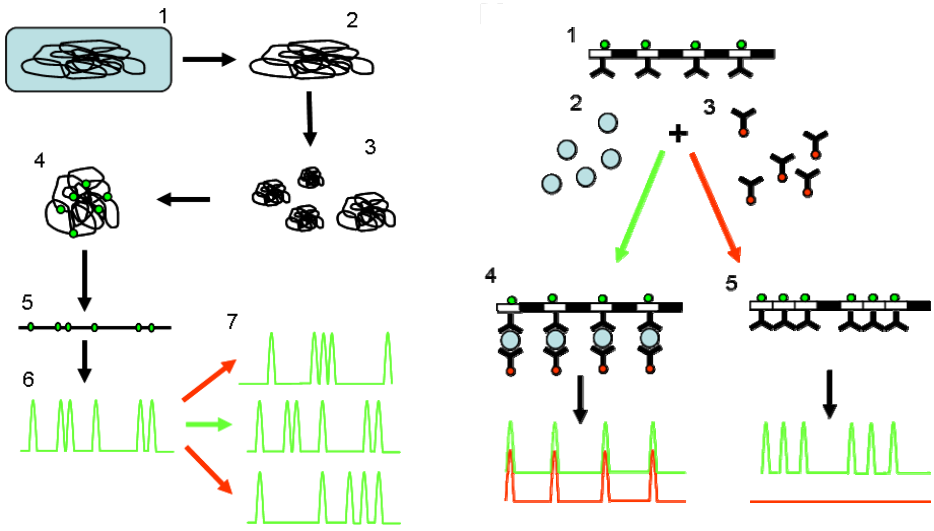
Clostridium botulinum and its toxin exemplify the requirement for broad-spectrum, multimodal pathogen detection in clinical diagnostics and food safety testing (Cheng et al., 2008; Sobel et al., 2004). The potential for weaponisation of botulinum toxin also necessitates rapid, sensitive, and specific diagnostics to detect and identify potential biowarfare threats (Arnon et al., 2001). Seven distinct serotypes of botulinum toxin arise from multiple distinct strains of *clostridia* (Fang et al., 2010; Peck, 2009; Raphael et al., 2010a, 2010b). Specific identification of the intoxicating serotype is required for effective selection of therapeutic antitoxin (Arnon et al., 2001). Rapid, strain-specific bacterial identification as well as multiplex antigen detection capabilities are not easily combined in a single platform. Here we present two technologies that leverage the enormous informational content of long (60 kb to 350 kb) DNA molecules for the strain-specific identification of bacteria and for multiplex detection of small analytes. These techniques are genome sequence scanning (GSS) (Protozanova et al., 2010) and digital DNA (DD) multiplex antigen detection (Burton et al., 2010), respectively.

Figure 1 Flow diagrams for (a) GSS and (b) DD



(a) (b)

Note: Process details are provided in text.

For bacterial samples (with a high intrinsic genomic content) GSS allows for direct strain-specific identification even from complex mixtures [Figure 1(a)] (Meltzer et al., 2011; Protozanova et al., 2010; White et al., 2009). A bacterial sample (1) is loaded into a membrane-based reaction chamber in which genomic DNA is released and isolated from other cellular components using radial field-flow fractionation (2) (Mollova et al., 2009). The isolated DNA is subsequently digested with restriction enzymes (3) and tagged with sequence-specific fluorescent peptide-nucleic acid tags (4). The finished sample consists of purified and labelled DNA, hundreds of kilobases in length. This process is comparable with traditional gel-plug DNA preparation for pulsed field gel electrophoresis (PFGE), but takes less than three hours compared to potentially multiple days for gel preparation (Mollova et al., 2009; Nevas et al., 2005).

The fluorescent-labelled DNA is subsequently transferred to a microfluidic device that extends each molecule of DNA into a fully linearised conformation (5), thus revealing the specific location of the fluorescent tags along the molecule (Chan et al., 2004; Larson et al., 2006). These fluorescent labels are detected using a custom fibre-coupled confocal microscope. The spatial distribution of fluorescent tags (6) can then be compared to a database of potential matching templates, which can be generated either theoretically from whole-organism sequence or experimentally from isolated bacterial culture (7). The detected fluorescence patterns are highly specific to individual strains of bacteria. High-throughput acquisition of hundreds of thousands of individual DNA molecules therefore allows for very high confidence in bacterial strain typing or detection of minor bacterial populations in complex mixtures of bacteria. The probes used in GSS are not organism-specific, but rather decorate all genomes with distinct spatial distributions determined by the underlying genetic sequence. This allows for truly broad spectrum bacterial identification and typing with a common reagent set, obviating the need for organism specific chemistries.

The efficacy of GSS at strain typing closely related bacterial cultures has recently been demonstrated (White et al., 2009). The genetic similarity of 71 strains of *Staphylococus aureus*, including sequenced, strain-typed, and clinical isolates were compared using GSS. The phylogenetic relatedness of the bacterial strains was readily determined, with results comparable to PFGE, but with faster, automated sample preparation and greater sample-to-sample reproducibility. GSS also allows for simultaneous detection of multiple target organisms from a single complex mixture of bacteria. This also has recently been demonstrated, where *Escherichia coli* and *Staphyloocus epidermidis* were detected in a complex mixture of nine additional bacteria at 1% and 4% of total cell count respectively (Meltzer et al., 2011).

For analytes with low genomic content, such as peptide toxins or small viruses, the DD multiplex antigen detection assay provides a detection strategy that is completely compatible with the sample preparation and detection hardware used for GSS [Figure 1(a)] (Burton et al., 2010). In this assay, long (125–185 kb) engineered DNA molecules are utilised as binary encoded fluorescent labels for a multiplex antigen-antibody sandwich assay. A given assay can include multiple specific antibodies, each attached to a uniquely encoded DNA label (1). Binding of the target antigen (2) to the DNA label permits coupling of secondary antibody labelled with a different fluorophore (3) to the DNA-antibody-antigen complex. When the prepared sample is processed through the microfluidic detection instrument, a specific fluorescence pattern is observed based on the binary encoding of the DNA label (4). If the target antigen is present, that fluorescence pattern is replicated in the second fluorescence channel. Otherwise, no secondary fluorescence is detected (5). The binary signal encoding of the DNA label allows for the potential detection of thousands of individual analytes in a single assay. The DD assay yields sub-picomolar sensitivity. Its multiplex detection capabilities have been demonstrated by the simultaneous specific detection of botulinum toxoid and MS2 virus in a single sample (Burton et al., 2010). Because the antigen-binding tag is itself a long DNA molecule, the sample preparation and detection processes for DD can be performed on the same instrumentation as GSS. Microfluidic devices designed for simultaneous processing of GSS and DD assays have previously been described (Meltzer et al., 2011).

In the management of botulinum poisoning, either from accidental food borne contamination or from intentional distribution, serotype specific identification of the toxin and strain specific identification of the bacterium is critical for appropriate therapeutic intervention, epidemiological tracking of the outbreak, and forensic analysis of the nature of the incident (Arnon et al., 2001). GSS has the capability to directly provide strain-specific identification of bacteria even from complex samples and from a variety of sample types. The same instrumentation is capable of processing human clinical samples as well as food or environmental samples. This rapid and sensitive assay may provide sufficient information to identify the likely toxin serotype by association of toxins to specific strains of bacteria. The standard for serotype identification of toxin, however is based on antigen recognition (Arnon et al., 2001). DD provides a convenient, sensitive, and multiplex-capable platform in the GSS enabled laboratory. The combination of GSS and DD technologies therefore provide an important suite of tools for botulinum research and testing, but with potentially broad-reaching applicability in the fields of human clinical diagnostics, food safety testing, and environmental monitoring.

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