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Cost-Effective, Multiplex Detection of CDC Urgent Threat Multi-Drug Resistance Markers and Toxigenic *Clostridioides difficile* with ChromaCode's HDPCR™ Multi-Drug Resistance Panel RUO

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Introduction

The CDC classifies carbapenem resistant Enterobacteriaceae (CRE), carbapenemase producing organisms (CPO), and toxigenic *Clostridioides difficile* as urgent antibiotic resistance threats. Various strategies have been adopted in order to combat spread of these infections, including expanded antimicrobial stewardship and infection prevention, and adoption of new diagnostics for better detection of colonization and characterization of multi-drug resistant infections. Effective surveillance and infection prevention strategies have been limited by the lack of molecular tests that have broad coverage, high enough test throughput, and are affordable enough to be a viable testing option without any consideration of reimbursement.

ChromaCode (Carlsbad, CA) is currently developing an extremely cost-effective, high-throughput and broadly multiplexed test for eight of the most common multi-drug resistance markers and toxigenic *C. difficile*. The test, called the HDPCR™ Multi-Drug Resistance (MDR) Panel Research Use Only (RUO), is a multiplex real-time PCR (qPCR) compatible with rectal and perirectal swabs in liquid transport and pure colonies. This study describes the design and development of MDR and the results from a series of analytical studies.

Materials/Methods

Method comparison, Inclusivity, and limit of detection (LOD) studies were performed to characterize the analytical performance of MDR. Testing was performed on the ABI 7500 Fast, ViiA 7, LC480 and QuantStudio 7. A combination of synthetic and clinical isolates were utilized for the studies. Results were analyzed on ChromaCode's cloud-based software ChromaCode Cloud.

ChromaCode Cloud

ChromaCode Cloud is a modern web application that provides rich visualizations of the assay results. The software is extremely user-friendly and enables streamlined results analysis and data management. The software can be run on just about any internet-enabled computer. Data upload from compatible qPCR instruments is automated and only takes a matter of seconds.

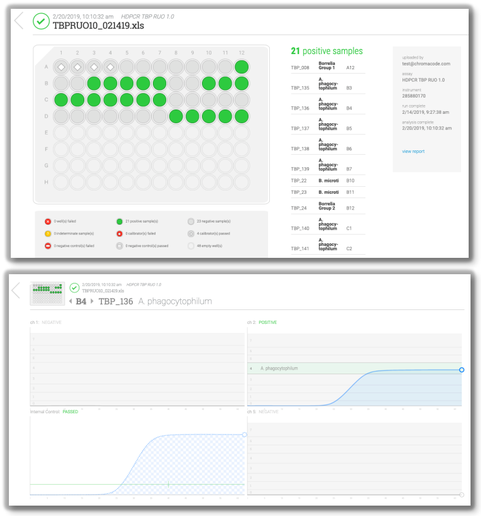

Figure 2. (Top) Plate Summary Page allows users to easily view plate results and navigate to individual wells; (Bottom) Well Summary Page allows users to view the results from a single sample

HDPCR™ Multi-Drug Resistance (MDR) Panel Overview

HDPCR Multiplexing Technology

ChromaCode's HDPCR multiplexing technology empowers the enormous, global installed base of real-time PCR (qPCR) and digital PCR (dPCR) instruments to perform multiplex testing for 5-50 targets at a very low cost with no instrument modifications required. Multiplexing with traditional qPCR/dPCR relies on differentiation of targets by color and is limited by the number of color channels available. HPDCR™ differs from this approach in that multiple targets can be encoded into a single color channel and be differentiated end-point signal intensity. MDR is the second test application using HDPCR multiplexing technology. MDR adopts a resilient coding scheme, which is designed to increase test sensitivity and specificity along with robustness in the face of new mutations by introducing an element of redundancy for most panel members.

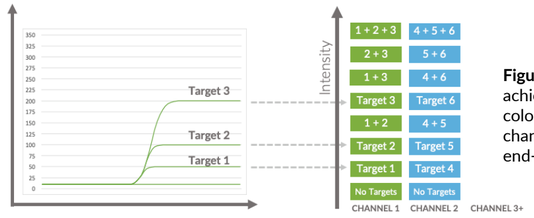

Figure 1. Depiction of how HDPCR achieves multiplexing within a single color channel and across multiple color channels by differentiating results by end-point signal intensity.

Table 1. Design of the Resilient Coded MDR

Level	Ch. 1 (FAM)	Ch. 2 (ATTO532)	Ch. 4 (ROX)	Ch. 5 (ATTO647)
Level 4	**IMP**	**vanA**	**vanA** or **tcdB** or **VIM**	**CTX-M** Grp 1, Grp 9)
Level 3	**OXA-48**	**KPC**		**NDM**
Level 2	**tcdB**	**MCR-1**	**KPC**	**VIM**
Level 1	**KPC**	**NDM**	**IC**	**OXA-48**

Results

Method Comparison Study. A total of 28 unique samples were tested in duplicate during the method comparison study (Table 2). Samples were obtained from Zeptometrix, ATCC, and the CDC (AR Bank specimens). For each specimen, 200 µL of sample was extracted to 50 µL nucleic acid extract before qPCR testing with MDR. ***Inclusivity Study.*** Inclusivity was assessed by testing of synthetic DNA targets and through in silico analysis. The inclusivity of each of the MDR targets is summarized in Table 3. ***LOD Study.*** A total of 144 samples were tested. Samples were generated by spiking synthetic DNA targets directly into TE buffer. The LOD for each of the 9 MDR targets was between 3-30 copies/reaction (Table 4). Some IMP alleles with mutations to primers and probes showed reduced sensitivity below 1,000 copies/reaction.

Table 2. Results from Method Comparison Study with MDR

Target	TP	TN	FP	FN	Sensitivity (95% Confidence Interval)	Specificity (95% Confidence Interval)
CTX-M	20	36	0	0	100% (80.0% - 100%)	100% (88.0% - 100%)
IMP	6	50	0	0	100% (51.7% - 100%)	100% (91.1% - 100)
KPC	6	49	1	0	100% (51.7% - 100%)	98.0% (88.0% - 99.9%)
MCR-1	0	56	0	0	N/A	100% (92.0% - 100%)
NDM	6	50	0	0	100% (51.7% - 100%)	100% (91.1% - 100)
OXA-48	6	50	0	0	100% (51.7% - 100%)	100% (91.1% - 100)
Toxigenic *C. difficile*	4	50	2	0	100% (39.6% - 100%)	96.2% (85.7% - 99.3%)
vanA	4	52	0	0	100% (39.6% - 100%)	100% (91.4% - 100%)
VIM	12	44	0	0	100% (69.9% - 100%)	100% (90.0% - 100%)
Total	**68**	**437**	**3**	**0**	**100% (93.3% - 100%)**	**99.3% (97.9% - 99.8%)**

Table 3. Results from Initial Inclusivity Study with MDR

Target	Total #	Inclusivity Strains / Subtypes
CTX-M-1 Grp	52 subtypes	1, 3, 10, 11, 12, 15, 22, 23, 28, 29, 30, 32, 33, 34, 36, 37, 42, 52, 54, 55, 57, 58, 60, 61, 62, 66, 68, 69, 71, 72, 79, 80, 82, 88, 96, 101, 103, 107, 108, 109, 114, 116, 117, 123, 132, 133, 136, 144, 169, 190, 207, 224
CTX-M-9 Grp	70 subtypes	9, 13, 14, 16, 17, 18, 19, 21, 24, 27, 38, 45 46, 47, 48, 49, 50, 51, 64, 65, 67, 73, 81, 83, 84, 85, 86, 87, 90, 93, 98, 99, 102, 104, 105, 106, 110, 111, 112, 113, 121, 122, 125, 126, 129, 130, 134, 137, 143, 147, 148, 159, 161, 168, 173, 174, 176, 177, 191, 192, 195, 196, 198, 199, 201, 213, 214, 215, 219, 221
IMP	66 subtypes	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 24, 25, 26, 27, 28, 29, 30, 33, 34, 35, 37, 38, 40, 41, 42, 48, 49, 51, 52, 53, 54, 55, 56, 58, 59, 60, 61, 62, 63, 64, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80
KPC	38 subtypes	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39
MCR-1	1 subtype	MCR-1
NDM	24 subtypes	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24
OXA-48	28 subtypes	48, 48b, 162, 163, 181, 199, 204, 232, 244, 245, 247, 370, 405, 416, 438, 439, 484, 505, 514, 515, 517, 519, 538, 546, 547, 566, 567
Tox. *C. difficile*	1 strain	1 strain tested
vanA	1 subtype	1 subtype tested
VIM	59 subtypes	1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 62

Table 4. Results from LOD Study with MDR

Target	LOD in TE Buffer (n = 20 / target)	LOD in Negative Stool (n = 4 / target)
CTX-M-1	10 copies / rxn	10 copies / rxn
CTX-M-2	10 copies / rxn	10 copies / rxn
IMP	3 copies / rxn	3 copies / rxn
KPC	3 copies / rxn	3 copies / rxn
MCR-1	10 copies / rxn	10 copies / rxn
NDM	10 copies / rxn	10 copies / rxn
OXA-48	3 copies / rxn	3 copies / rxn
Toxigenic *C. difficile*	30 copies / rxn	30 copies / rxn
vanA	3 copies / rxn	10 copies / rxn
VIM	3 copies / rxn	3 copies / rxn

Discussion

HDPCR is a novel technology that enhances multiplexing levels of traditional qPCR and dPCR instrumentation already in laboratories around the world. This technology will expand global access to multiplex testing by circumventing the high cost barriers currently tied to multiplex syndromic testing. MDR is designed in this spirit and provides a high-throughput and cost-effective testing for a wide variety of CDC urgent antibiotic resistance threats in a single reaction from rectal/perirectal swabs in liquid transport and pure isolates.