Development of a Respiratory Pathogen Panel with an Automated High Throughput System - BioCode MDx-3000

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Abstract

Introduction

Acute respiratory tract infections (ARIs) are the most common causes of childhood morbidity and mortality worldwide, which accounts for about 30% of all childhood deaths in the developing world. However in developed countries, ARIs cause enormous direct and indirect health costs[1]. Rapid identification of respiratory viruses can help in avoiding indiscriminate use of antibiotics as well as large healthcare costs. Using our proprietary Barcoded Magnetic Bead (BMB) technology, Applied BioCode is developing a multiplex molecular diagnostic assay for detection of respiratory pathogens in a high-throughput 96-well format. The BioCode Respiratory Panel is designed for detection of Influenza viruses (Influenza A: subtype H1N1 2009 Pandemic, H1 seasonal, H3, Influenza B), Parainfluenza viruses (type 1, 2, 3, 4A, 4B), RSV, human Metapneumovirus, Rhinovirus/ Enterovirus, Coronavirus (OC43, NL63, 229E, HKU1), Adenovirus and bacteria (*Bordetella pertussis*, *B. parapertussis*, *Mycoplasma pneumoiae, Chlamydophila pneumoniae,* and *Legionella pneumophila*).

1 Hinman, A. R. 1998. Global progress in infectious diseases control. Vaccine 16:1116-1121

Materials & Methods

Biocode MDx-3000 platform, an automated PCR and post-PCR sample handling and detection system in 96-well format was used for the study. Off board automated nucleic acid extraction system was used and pathogenic targets were amplified by one-step RT-PCR. PCR products were captured by target-specific probes coupled to barcoded magnetic beads (BMBs) and fluorescent signal was generated by incubation with a conjugate (Figure 1). Qualitative results were determined by Median Fluorescent Index (MFI) of fluorescent signals from analyte-specific BMBs (Figure 2).

Conclusions

The BioCode Respiratory Pathogen Panel run on BioCode MDx-3000 system, specifically and reproducibly detects viruses and bacteria known to cause upper respiratory infections. Combined, the automated system and molecular panel allows users to perform highly multiplexed molecular detection in a high-throughput, automated format with a simple workflow and minimal hands-on time.

- ❖ No cross reactivity was observed with organisms tested (Table 1).
- ❖ Preliminary LoD of the BioCode Respiratory Pathogen Panel is shown in Table 2.
- ❖ Inclusivity was tested with several relevant pathogens which were detected with high titer of pathogen (Table 3).
- ❖ Method comparison of 208 NPS showed 88% overall (and 92% without rhinovirus) positive agreement (Table 4 & 5).

The BioCode Respiratory Pathogen Panel is currently in development.

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Multiplex Respiratory Pathogen Panel

Viruses

- Influenza A
  - H1N1 2009pdm
  - H1 (seasonal)
  - H3
- Influenza B
- RSV A/B
- Human metapneumovirus
- Adenovirus
- Rhinovirus/ Enterovirus
- Parainfluenza virus type 1
- Parainfluenza virus type 2
- Parainfluenza virus type 3
- Parainfluenza virus type 4
- Coronavirus 229E
- Coronavirus HKU1
- Coronavirus NL63
- Coronavirus OC43

Bacteria

- *Bordetella pertussis*
- *Chlamydophila pneumoniae*
- *Mycoplasma pneumoniae*
- *Bordetella parapertussis*
- *Legionella pneumophila*

Barcoded Magnetic Bead (BMB) Technology

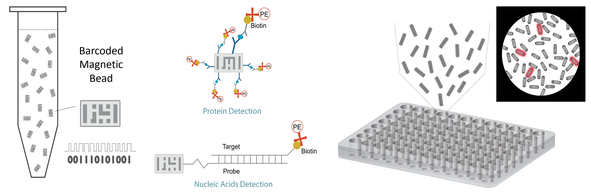


Figure 1. Barcoded Magnetic Beads (BMBs) are coupled to proteins or nucleic acids probes and used for target capture in microtiter plates. In the BioCode Respiratory Pathogen Panel, biotinylated PCR product is captured by target-specific nucleic acid probes coupled to BMBs then labeled by SA-PE for detection.

BioCode MDx-3000

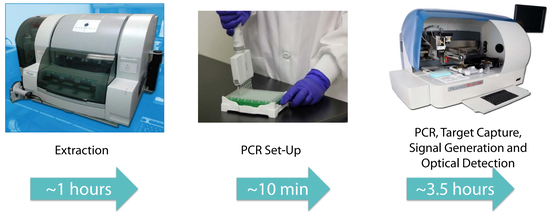
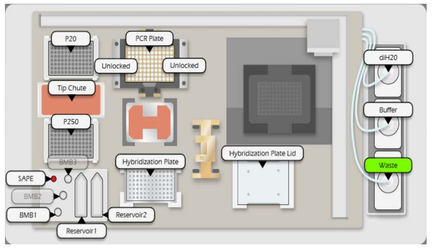


Figure 2. Workflow for BioCode Respiratory Pathogen Panel (top) and schematic for BioCode MDx-3000 deck layout (bottom). 192 samples in an 8 hour shift with minimal hands on time. Three different BioCode panels can be performed simultaneously in one plate.



Cross Reactivity Studies

Table 1. Microorganisms tested for cross reactivity

Bacteria and Fungi		
E. Coli 35328	*Pseudomonas aeruginosa*	*Candida albicans*
E.coli BAA-1431	*Staphylococcus aureus 3300*	*Klebsiella pneumoniae*
Haemophilus influenzae	*Staphylococcus epidermidis*	*Moraxella catarrhalis*
Lactobacillus acidophilus	*Streptococcus intermedius*	*Neisseria elongata*
Lactobacillus reuteri Strain	*Streptococcus pyogenes*	*Chlamydia trachomatis*
Neisseria gonorrhoeae	*Streptococcus salivarius*	*Lactococus lactic*
Acinetobacter baumannii		
Viruses		
Epstein-Barr Virus (EBV)	Bocavirus	Cytomegalovirus (CMV)
Herpes Virus	Mumps	

➢ No cross reactivity was observed with bacteria (≥10^6 CFU/mL), viruses (≥10^5 $TCID_{50}$/mL)

Preliminary Limit of Detection (LoD)

Table 2. Limit of Detection (LoD) for the BioCode Respiratory Pathogen Panel performed on the BioCode MDx-3000 system

Organism	Source	Preliminary LoD
Viruses		
Human Parainfluenza 1	BEI NR-48680	≤1.25 TCID50/mL
Human Parainfluenza 2	BEI NR-3229	≤10 TCID50/mL
Human Parainfluenza 3	Zeptometrix 0810016CF	≤1.25 TCID50/mL
Human Parainfluenza 4	Zeptometrix 0810060CF	≤1.25 TCID50/mL
Rhinovirus	Zeptometrix 0810012CFN	≤10 TCID50/mL
RSV A & B	Zeptometrix 0810040ACF	≤1.25 TCID50/mL
Coronavirus OC43	Zeptometrix 0810024CF	≤1.25 TCID50/mL
Coronavirus 229E	Zeptometrix 0810229CF	≤1.25 TCID50/mL
Coronavirus NL63	Zeptometrix 0810228CF	≤1.25 TCID50/mL
Coronavirus HKU1	IDT- Ultramer	≤100 copies/mL
Human Metapnuemovirus	Zeptometrix 0810161CF	≤100 TCID50/mL
Influenza A H3	Zeptometrix 0810252CF	≤10 TCID50/mL
Influenza A H1 subtype	Zeptometrix 0810036CF	≤1.25 TCID50/mL
Influenza A H3 subtype	Zeptometrix 0810252CF	≤10 TCID50/mL
Influenza A H1N1 2009pdm	IDT- Ultramer	≤100 copies/mL
Influenza B	Virapur C1320H	≤10 TCID50/mL
Adenovirus	Zeptometrix 0810050CF	≤1 TCID50/mL
Bacteria		
Bordetella pertussis	Zeptometrix 0801460	≤1.25 CFU/mL
Bordetella parapertussis	Zeptometrix 0801461	≤2.5 CFU/mL
Chlamydophila pneumoniae	ATCC VR-1360D	≤100 copies/mL
Legionella pneumoniae	ATCC 33152D-5	≤100 copies/mL
Mycoplasma pneumoniae	Zeptometrix 0801579	≤1.25 CFU/mL

Method Comparison Study

Table 5. Clinical Agreement: BioCode RP Panel vs Comparator

Overall Agreement		GenMark RVP		
		Pos	Neg	Total
BioCode RP Panel	Pos	165	0	165
	Neg	23	20	43
	Total	188	20	208

Positive Agreement	88%
Negative Agreement	100%

Inclusivity Study

Table 3. Organisms detected for Inclusivity of the BioCode Respiratory Pathogen Panel

Organisms Detected	
Influenza A/Denver/1/1957	Coronavirus 229E
Influenza A/Fort Monmouth/01/1947	Coronavirus NL63
Influenza A/New Jersey/8/1976	Coronavirus OC43
Influenza A/Taiwan/42/06	Adenovirus type 1
Influenza A/Singapore/63/04	Adenovirus type 3
Influenza A/ Aichi/2/68	Adenovirus type 4
Influenza A/Port Chalmers/1/1973	Adenovirus type 5
Influenza A/Switzerland/9715293/2013	Adenovirus type 7A
Influenza A/Wisconsin/15/2009 H3N2	Adenovirus type 8
Influenza A/PR/8/34	Adenovirus type 14
Influenza A/Beijing/262/95	Adenovirus type 37
Influenza A/Brisbane/59/2007 H3N2	Respiratory Syncytial Virus type A
Influenza A/Urugay/706/07	Respiratory Syncytial Virus type B
Influenza A/New Caledonia/20/99	Rhinovirus Type B14
Influenza A/Solomon Islands/03/3006	Metapnuemovirus 3 Type B1
Influenza A/H3N2/Hong Kong	Metapnuemovirus 20 Type A
Influenza A/H3N2/Victoria/3/1975	Metapnuemovirus 316 Type A
Influenza B/Maryland/1/59	Metapnuemovirus 213 Type A
Influenza B/PHUKET/3073/2013	Metapnuemovirus 9 Type A
Influenza B/GL/1739/55	Metapnuemovirus 16 Type A
Influenza B/Hong Kong/5/1972	Enterovirus Type 68
Influenza B/Brisbane/60/2008	Coxsackivirus Type A9
Influenza B/Florida/04/06	Echovirus Type 6
Influenza B/Christ Church	Echovirus Type 9
Influenza B/Sydney/507/2006	Echovirus Type 30
Influenza B/OHIO/01/2005	Echovirus Type 11
Influenza B/Malaysia/2506/04	*Bordetella parapertusis A747*
Influenza B/Texas/06/2011	*Bordetella pertusis E431*
Influenza B/Nevada/03/2011	*Bordetella pertusis A639*

Method Comparison Study

Table 4. Detection of target pathogens: BioCode Respiratory Panel vs previously reported test results

Target Pathogens	Positive Results reported by	
	BioCode RP Panel	GenMark RVP
Influenza A (not subtyped)	20	20
Influenza A H3	11	11
Influenza A H1N1 2009pdm	14	18
Influenza A H1	2	2
Influenza B	28	31
RSV	38	42
Rhinovirus/ Enterovirus	17	27
Metapneumovirus	20	20
Parainfluenza virus	15	17
Negative NPS	20	20