

Abstract

Introduction

Respiratory infections caused by a variety of virus and bacteria, are a major cause of hospitalization and often produce similar presentations. Specific diagnosis therefore relies on laboratory investigation. General pathogen diagnosis was performed via pathogen isolation in cell culture and immunofluorescent assays. However in recent times, reverse transcriptase PCR is used as a sensitive and specific alternative for detection. 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 Pathogen Panel (RPP) 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*, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*).

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

Materials & Methods

BioCode® MDx 3000, an automated PCR, 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® RPP on BioCode® MDx 3000 system specifically and reproducibly detects 17 viruses and 3 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® RPP is shown in Table 2.
- ❖ Inclusivity was tested with several relevant pathogens which were detected with high titer of pathogen (Table 3).
- ❖ Method comparison using 307 NPS showed 96% positive agreement (Table 4 & 5) with an IVD comparator.

BioCode® Respiratory Pathogen Panel

Viruses

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

Bacteria

Bordetella pertussis
Chlamydia pneumoniae
Mycoplasma pneumoniae

Barcoded Magnetic Bead (BMB) Technology

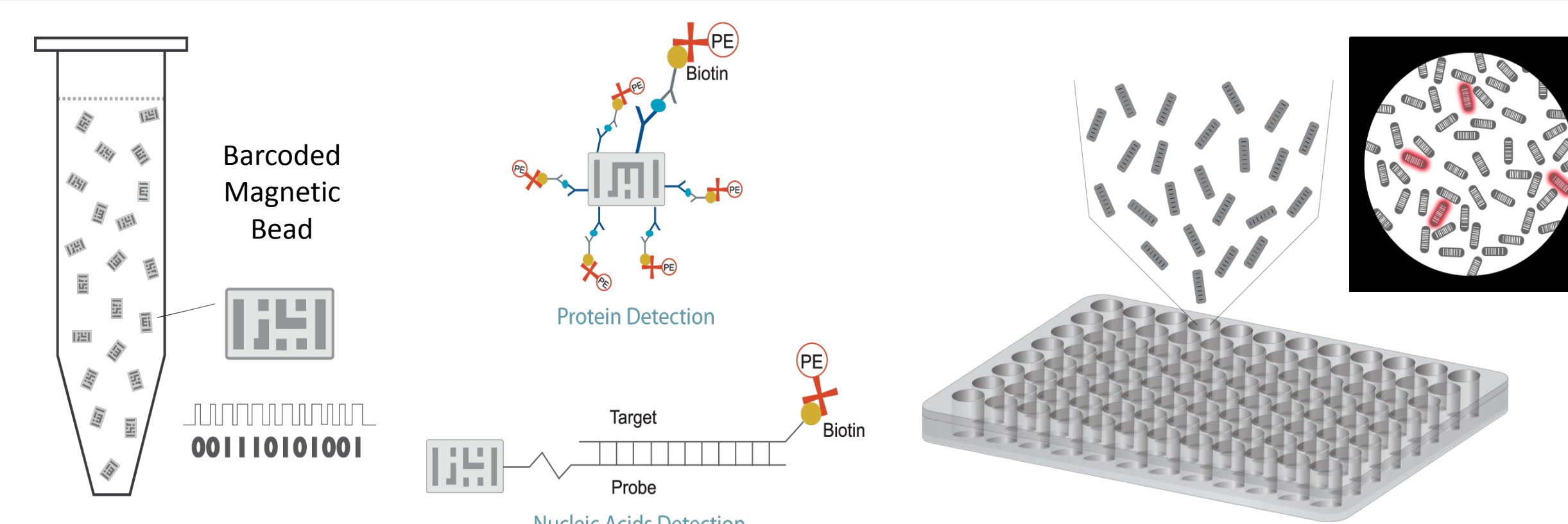
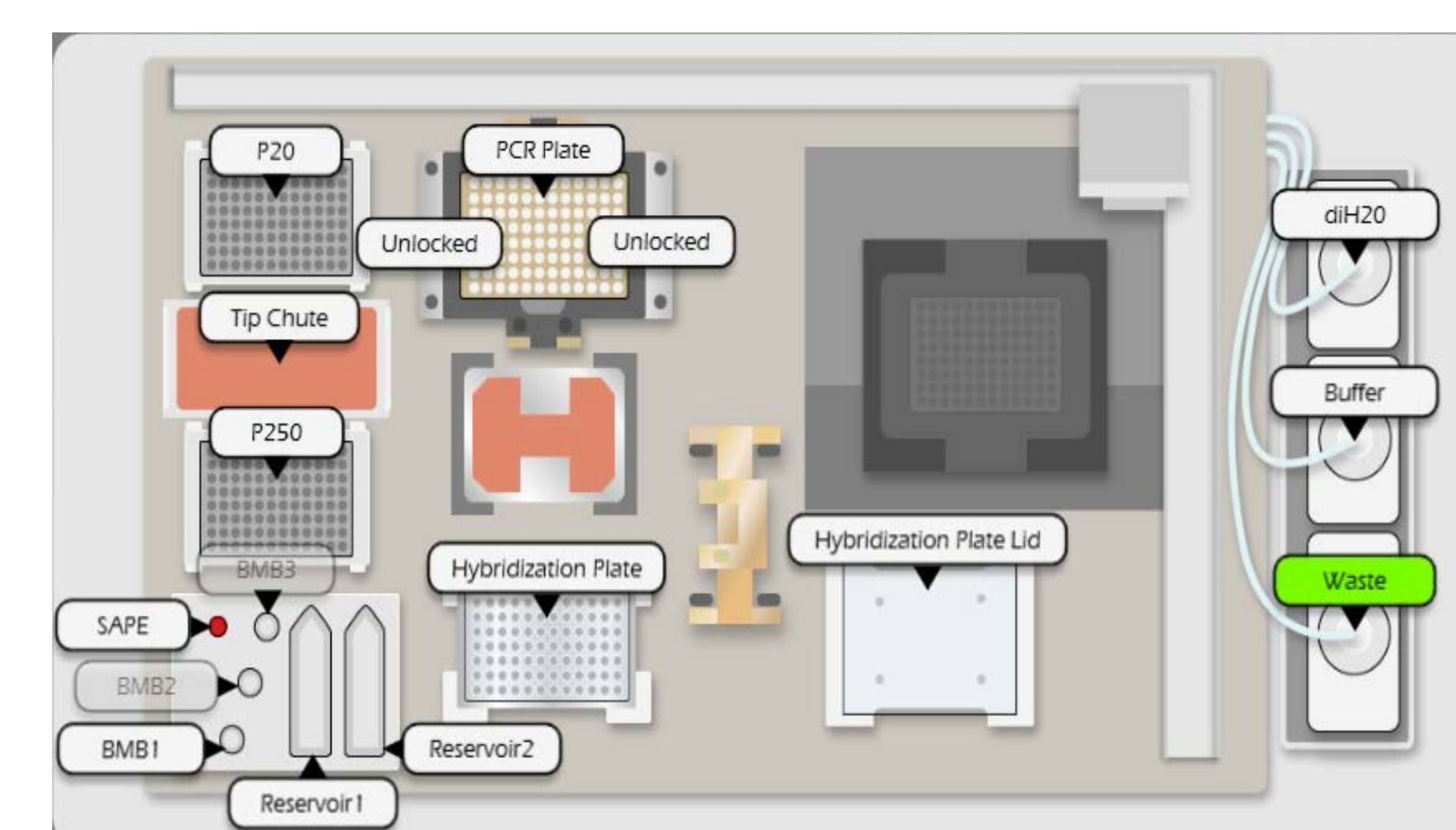


Figure 1. Barcoded Magnetic Beads (BMBs) are coupled to proteins or nucleic acids probes and used for target capture in microtiter plates. For BioCode® RPP, biotinylated PCR products are captured by target-specific nucleic acid probes coupled to BMBs and then labeled by SA-PE for detection.

BioCode® MDx 3000



Figure 2. Workflow for BioCode® RPP (top) and schematic for BioCode® MDx 3000 deck layout (bottom). 192 samples in an 8 hour shift with minimal hands on time. This system is designed to run 3 different BioCode® panels simultaneously in one run.



Cross Reactivity Studies

Table 1. Microorganisms tested for Cross Reactivity

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

➤ No cross reactivity was observed with bacteria ($\geq 10^6$ CFU/mL), viruses ($\geq 10^5$ TCID₅₀/mL)

Preliminary Limit of Detection (LoD)

Table 2. Limit of Detection (LoD) for the BioCode® RPP performed on the BioCode® MDx 3000 system

Organism	Source	Preliminary LoD
Viruses		
Human Parainfluenza 1	Zeptomatrix 0810014CF	≤ 1.25 TCID ₅₀ /mL
Human Parainfluenza 2	ATCC VR92	≤ 10 TCID ₅₀ /mL
Human Parainfluenza 3	Zeptomatrix 0810016CF	≤ 1.25 TCID ₅₀ /mL
Human Parainfluenza 4	Zeptomatrix 0810060CF	≤ 1.25 TCID ₅₀ /mL
Rhinovirus	Zeptomatrix 0810012CFN	≤ 10 TCID ₅₀ /mL
RSV A & B	Zeptomatrix 0810040ACF	≤ 1.25 TCID ₅₀ /mL
Coronavirus OC43	Zeptomatrix 0810024CF	≤ 1.25 TCID ₅₀ /mL
Coronavirus 229E	Zeptomatrix 0810229CF	≤ 1.25 TCID ₅₀ /mL
Coronavirus NL63	Zeptomatrix 0810228CF	≤ 1.25 TCID ₅₀ /mL
Coronavirus HKU1	IDT- Ultramer	≤ 100 copies/mL
Human Metapneumovirus	Zeptomatrix 0810161CF	≤ 100 TCID ₅₀ /mL
Influenza A H3	Zeptomatrix 0810252CF	≤ 10 TCID ₅₀ /mL
Influenza A H1 subtype	Zeptomatrix 0810036CF	≤ 1.25 TCID ₅₀ /mL
Influenza A H3 subtype	Zeptomatrix 0810252CF	≤ 10 TCID ₅₀ /mL
Influenza A H1N1 2009pdm	Zeptomatrix 080109CFN	≤ 10 TCID ₅₀ /mL
Influenza B	Virapur C1320H	≤ 10 TCID ₅₀ /mL
Adenovirus	Zeptomatrix 0810050CF	≤ 1 TCID ₅₀ /mL
Bacteria		
<i>Bordetella pertussis</i>	Zeptomatrix 0801460	≤ 1.25 CFU/mL
<i>Mycoplasma pneumoniae</i>	Zeptomatrix 0810579	≤ 10 CFU/mL
<i>Chlamydia pneumoniae</i>	ATCC VR-1360	≤ 10 CFU/mL

Method Comparison Study

Table 5. Clinical Agreement: BioCode® RPP vs Comparator

Overall Agreement	eSensor RP		
	Pos	Neg	Total
BioCode® RPP	Pos: 307	Neg: 0	Total: 307
	Neg: 12	20	32
	Total: 319	20	339
	Positive Agreement		96%
	Negative Agreement		100%

Inclusivity Study

Table 3. Microorganisms detected for Inclusivity of the BioCode® RPP

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/Uruguay/706/07	Respiratory Syncytial Virus type B
Influenza A/New Caledonia/20/99	Rhinovirus Type B14
Influenza A/Solomon Islands/03/3006	Metapneumovirus 3 Type B1
Influenza A/H3N2/Hong Kong	Metapneumovirus 20 Type A
Influenza A/H3N2/Victoria/3/1975	Metapneumovirus 316 Type A
Influenza B/Maryland/1/59	Metapneumovirus 213 Type A
Influenza B/Phuket/3073/2013	Metapneumovirus 9 Type A
Influenza B/GL/1739/55	Metapneumovirus 16 Type A
Influenza B/Hong Kong/5/1972	Enterovirus Type 68
Influenza B/Brisbane/60/2008	Coxsackievirus 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	<i>Bordetella pertussis</i> A639
Influenza B/Texas/06/2011	<i>Bordetella pertussis</i> E431
Influenza B/Nevada/03/2011	

Method Comparison Study

Table 4. Comparison of BioCode® RPP results vs Comparator

Target Pathogens	Positive Results reported by	
	BioCode® RPP	eSensor RP
Influenza A (not subtyped)	17	17
Influenza A H3	18	20
Influenza A H1N1 2009pdm	18	20
Influenza A H1	2	2
Influenza B	20	20
RSV	20	20
Rhinovirus/ Enterovirus	18	20
Metapneumovirus	27	28
Parainfluenza virus	74	75
Coronavirus	30	33
Adenovirus	18	20
<i>B. pertussis</i>	20	20
<i>M. pneumoniae</i>	14	14
Negative NPS	20	20