DETECTION OF RESPIRATORY VIRUSES BY MULTICODE®-PLx USING BARCODED MAGNETIC BEADS

T S Ramasubramanian[1], Gerda Harms[1], Gao Chen[2], Elizabeth K. Mulligan[1], Kelly Homb[1], and Scott C. Johnson[1]
[1]EraGen Biosciences, Madison, WI and [2]Applied BioCode, Santa Fe Springs, CA

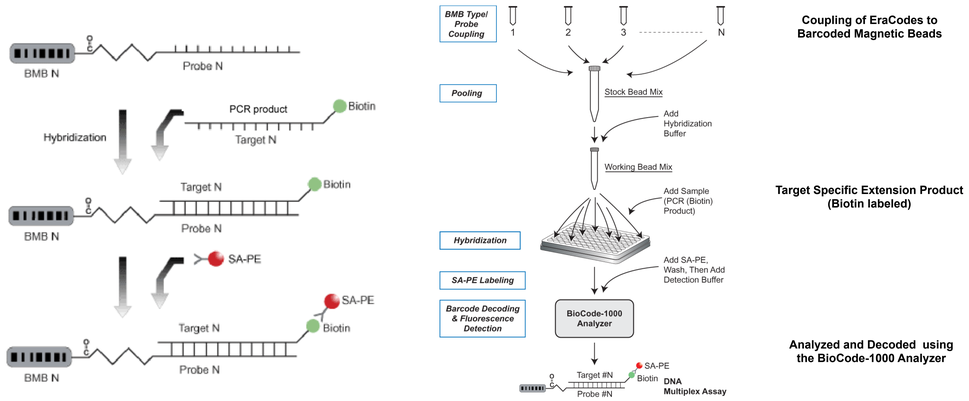
#T5

Abstract

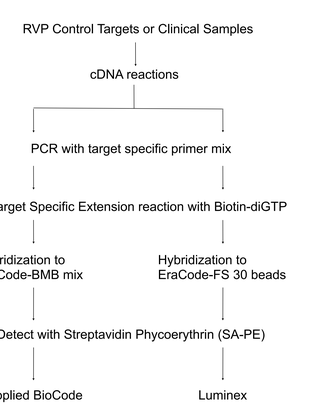
We have used the MultiCode®-PLx assay in conjunction with the Barcoded Magnetic Bead technology for the detection of respiratory virus targets. The MultiCode-PLx assay allows for the analysis of multiple nucleic acid targets in a single reaction, by utilizing an expanded genetic alphabet to label and capture PCR-amplified viral nucleic acids. The analytes were captured via sequence tags (EraCodes) immobilized on bar-coded magnetic beads and analyzed using the BioCode-1000 analyzer. Optically bar-coded beads can be discriminated and the surface fluorescence signal measured for each bar-coded bead representing an interaction between the amplified nucleic acid target and the bar-coded bead probe, indicating the presence of the specific viral target in the sample.

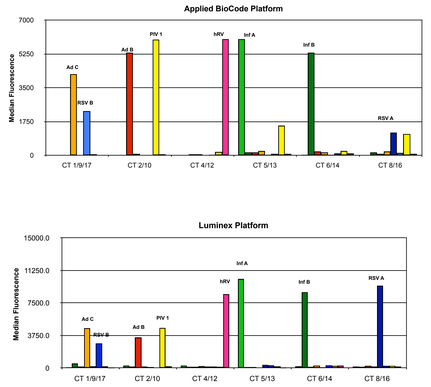
The MultiCode-PLx assay was performed using a subset of a previously developed Respiratory Virus Panel (RVP) that included Influenza virus (FluA and FluB), Respiratory Syncytial virus (RSVA and RSVB), Adenovirus (B, C), Rhinovirus (HRV), and Parainfluenza virus (PIV1). Targets representing eight of the viruses were amplified and labeled using a multiplex primer pool. The resulting extension products were hybridized to bar-coded magnetic beads. The beads were then analyzed on the BioCode-1000 analyzer and the fluorescent signal emitting from the beads quantitated. For comparison, the labeled extension products from the same eight targets were also analyzed on the Luminex 1000 IS™ system (Luminex Corporation, Austin, TX). All the viral targets were specifically detected by the both the Applied BioCode as well as the Luminex systems.

Barcoded Magnetic Bead Technology



RVP Controls on Applied BioCode and Luminex Platforms

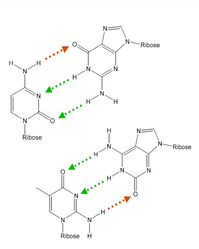




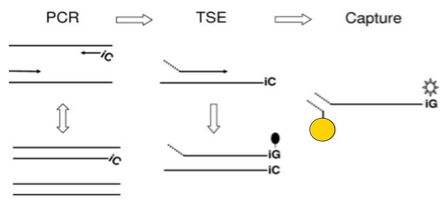
MultiCode-PLx Technology

MultiCode - Base Pairing

MultiCode-PLx uses an additional nucleobase pair constructed from the complementary nucleobases 2-deoxy-isoguanosine (isoG) and 5-Me-iso-cytosine (isoC). These nucleobases specifically recognize each other, but with a different pattern of hydrogen bondings. The pattern of hydrogen bond donors and acceptors is rearranged for the MultiCode base pair between isoC and isoG (bottom) compared to a standard cytosine and guanosine (top) base pair.



Steps of the MultiCode-PLx system[1]



Step-1: Multiplex PCR

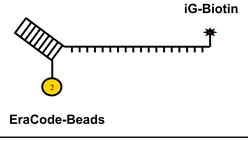
Target regions are amplified using primers that contain an Isobase resulting in PCR amplicons with an iC at the 5' end for subsequent site-specific labeling.

Step-2: Target Specific Extension (TSE)

Target-specific extenders are site-specifically labeled with Biotin-diGTP when the correct amplicon is present.

Step-3: Capture and Decoding

The biotin labeled target-specific extension products are captured on EraCode-modified BMBs through hybridization of each tag to its complement. The biotin is detected using Streptavidin-Phycoerythrin and the fluorescent signal associated with each BMB is analyzed and decoded using the BioCode-1000 analyzer.



Materials and Methods

Controls and Clinical Specimens:

A subset of previously developed Respiratory Virus Panel controls that included Influenza virus (Inf A and Inf B - matrix M gene), Respiratory Syncytial virus (RSV A and RSV B - Fusion F gene), Adenovirus (Ad B and Ad C - hexon gene), Parainfluenza virus (PIV1 - HN gene), and Rhinovirus (hRV - 5' non-coding region) that represent cloned region of viral nucleic acid[2] and a set of clinical specimens from a retrospective study were used.

Nucleic acids were isolated from clinical specimens using Roche MagNA Pure LC instrument. 200 µL of each specimen was extracted using the MagNA Pure Total Nucleic Acid Kit according to the manufacturer recommended protocol and eluted in a final volume of 100 µL.

EraCodes:

EraCodes are 8 base sequence tags that contain a mixture of natural and 2-3 MultiCode bases. EraCodes are complementary to the specific tag sequences that are part of the target-specific extenders that are used in TSE reactions.

Barcoded Magnetic Beads (BMB):

Barcoded Magnetic Beads are 100 X 30 x 6 µm beads formed by combining biocompatible polymer with paramagnetic material. These optically bar-coded beads are functionalized with carboxyl groups on the surface for conjugation to amine-containing oligonucleotides. The BMBs' barcode patterns give a high-contrast transmitted signal allowing the barcode to be decoded and identified.

Coupling of EraCodes to BMBs:

For each coupling reaction, a solution of 3' amine-modified EraCode DNA oligonucleotides was coupled to ~5000 carboxylated BMBs via EDC mediated reaction. Beads from individual coupling reactions were pooled and re-suspended in 500 µL of PBS to generate 8-EraCode coupled BMBs (~500 beads each).

MultiCode-PLx Reactions using Luminex Beads:

For comparison, the control targets and clinical samples were also assayed using the Luminex microspheres and read on the Luminex[100] IS™ system. The Biotin labeled target specific extended products were captured by EraCode coupled Luminex microspheres and detected using Streptavidin-Phycoerythrin (SA-PE).

MultiCode-PLx Reactions with

Target Amplification:

Nucleic acid from the clinical specimens or the control targets were reverse transcribed using random hexamers and AMV reverse transcriptase. The cDNA from the RT step was amplified using target specific primer sets via PCR. For each viral target, one target-specific primer contained a single iC at the 5' end for subsequent site-specific labeling with iso-GTP. The cycling profile for the PCR was 2m@95˚C (10s@ 95˚C, 30s@ 55˚C, 30s@ 72˚C)*30 cycles.

Target-Specific Extension:

Target-specific extension reactions were performed on PCR amplified products using Target-specific extenders that contain 3' bases that are target-specific and 5' bases that are complementary to the EraCodes. Target-specific extension reaction involved incorporation of biotin-diGTP and was done using the cycling parameters: 30s @ 95˚C, (10s @ 95˚C, 2m @ 65˚C)*10, 5m @ 65˚C

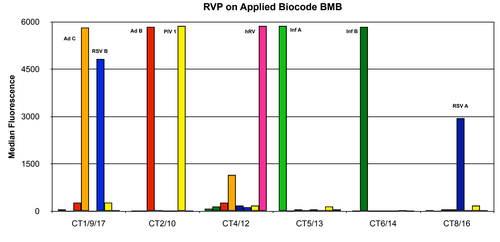
Capture and Detection of EraCode Labeled BMBs:

Biotin labeled target specific extended products were captured by EraCode coupled BMB beads via a 10 minute hybridization reaction at room temperature and detected using Streptavidin-Phycoerythrin (SA-PE). The beads were washed with 0.1% Tween in PBS, re-suspended in Detection buffer, decoded and analyzed using the BioCode-1000 analyzer.

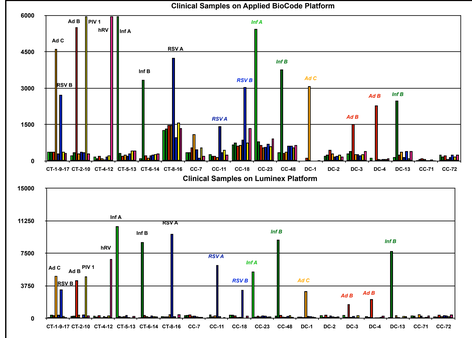
Decoding and measurement of fluorescent signal:

The EraCode coupled BMBs that are complementary to specific tags on the extenders were hybridized to the labeled target specific extension product and analyzed on the BioCode-1000 analyzer. The magnetic beads were decoded and the associated Phycoerythrin fluorescence emitting from the BMBs detected by a CCD camera and expressed as median fluorescence units.

RVP controls on Applied BioCode Instrument



Clinical Samples on Applied BioCode and Luminex Platforms



Specimen	Known call	MultiCode-PLx - Applied BioCode	MultiCode-PLx - Luminex
CC-7	RSV A	Neg	Neg
CC-11	RSV A	RSV A	RSV A
CC-18	RSV B	RSV B	RSV B
CC-23	Inf A	Inf A	Inf A
CC-48	Inf B	Inf B	Inf B
DC-1	Ad	Ad C	Ad C
DC-2	Ad	-	-
DC-3	Ad	Ad B	Ad B
DC-4	Ad	Ad B	Ad B
DC-13	Inf B	Inf B	Inf B
CC-71	Neg	Neg	Neg
CC-72	Neg	Neg	Neg

Results and Conclusions

1. MultiCode-PLx assay in conjunction with the Barcoded Magnetic Bead technology has been successfully used for the detection of respiratory virus targets.
2. The MultiCode-PLx assay detected all the 8 RVP controls tested in this study, both on the Applied BioCode platform and the Luminex platform.
3. Performance of the MultiCode-PLx assay using Barcoded Magnetic Beads on the Applied BioCode platform was equivalent to the MultiCode-PLx assay on the Luminex platform.
4. The MultiCode-PLx assay using Barcoded Magnetic Beads on the BioCode-1000 analyzer offers a robust, low maintenance technology platform for multiplexed molecular testing.

References

1. Nolte, F., D. Marshall, C. Rasberry, S. Schievelbein,G. Banks, G. Storch, M. Arens, R. Buller, and J. Prudent. 2007. MultiCode-PLx System for Multiplexed Detection of Seventeen Respiratory Viruses. 2007. J. Clin. Microbiol. 45:2779-2786.
2. Lee, W. K. Grindle, T. Pappas, D. Marshall, M. Moser, E. Beaty, P. Shult, J. Prudent, and J. Gern. High-throughput, sensitive, and accurate multiplex PCR-microsphere flow cytometry system for large-scale comprehensive detection of respiratory viruses. 2007. J. Clin. Microbiol. 45:2626-34.

Acknowledgements

We would like to thank Nik Voss for technical assistance.

26th Annual Clinical Virology Symposium
Daytona Beach, FL April 25 - 28, 2009