# A Novel High Plex Analysis System with Molecular and Immunoassay Capabilities

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## Introduction

Assay multiplexing provides an attractive solution for improving laboratory efficiency since, unlike traditional 'one tube – one result' methods, a multiplex assay can provide results for multiple targets in a single reaction, saving time, labor, reagents, samples, and thereby reducing overall costs. PlexBio has developed a novel high-plex platform that is compatible with both molecular and immunoassay formats to serve this critical laboratory need. PlexBio's πCode™ MicroDisc technology is currently the largest multiplexing platform commercially available, capable of generating over 16,000 circular image patterns. Each image pattern corresponds to an individual target which can be detected simultaneously via the advanced optical imaging and fluorescence reading capabilities of the PlexBio™ 100. The PlexBio™ 100 analyzer works in conjunction with the IntelliPlex™ 1000 processor for the hybridization of amplicon, MicroDisc washing and fluorescence labelling. Potentially any assay test can be developed using πCode™ technology platform. We present here several examples across a range of applications, including an EGFR single gene mutation test with 40 individual targets, a high complexity Lung Cancer Panel assay containing >60 widely used single gene mutations and gene re-arrangement targets, infectious disease testing for HCV and HPV genotyping, immunological measurements for the traditional TORCH panel and a highly sensitive general food microbiology testing panel. The broad applicability of the platform can serve a range of needs. By using this higher order multiplexing platform, PlexBio is able to offer highly sensitive and specific results for both immuno and molecular diagnostic formats from very low input sample volumes – saving laboratories time and labor.

## Methods

**Figure 1. Figure illustrating the workflow for multiplex assays using πCode™ technology platform, and its compatibilities for both molecular and immunoassay applications.**

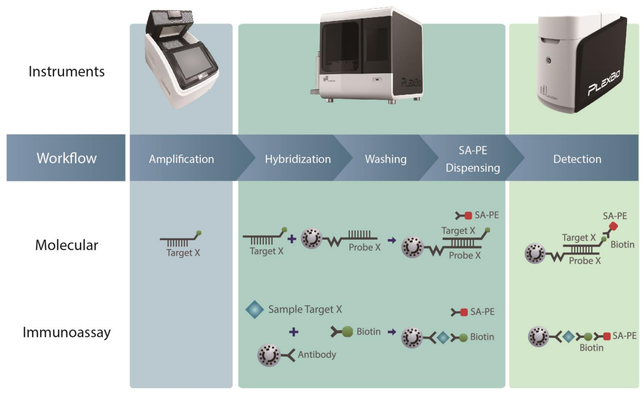



**Figure 2. Each πCode™ corresponds to an individual target which can be detected simultaneously via the advanced optical imaging and fluorescence reading capabilities of the PlexBio™ 100.**

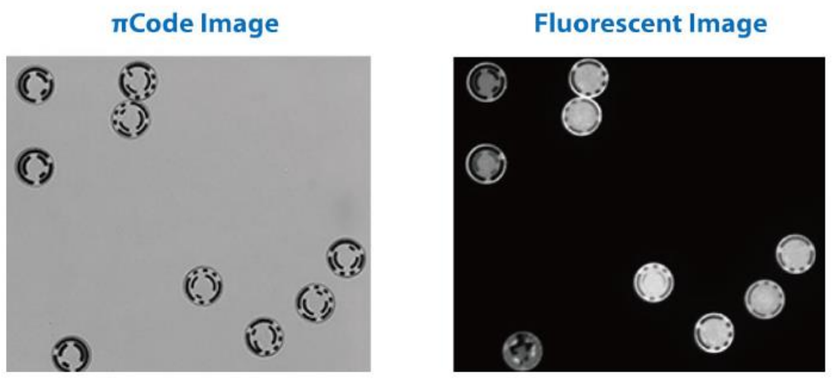
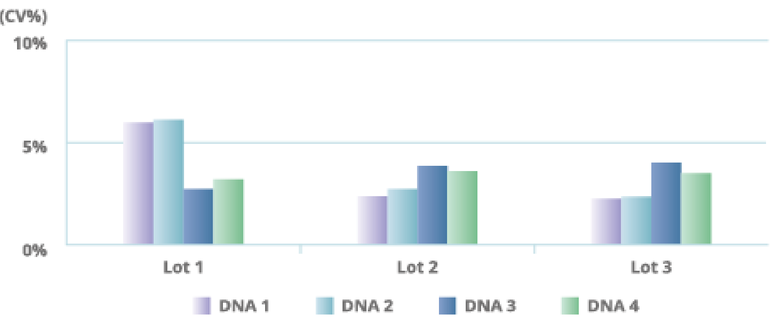
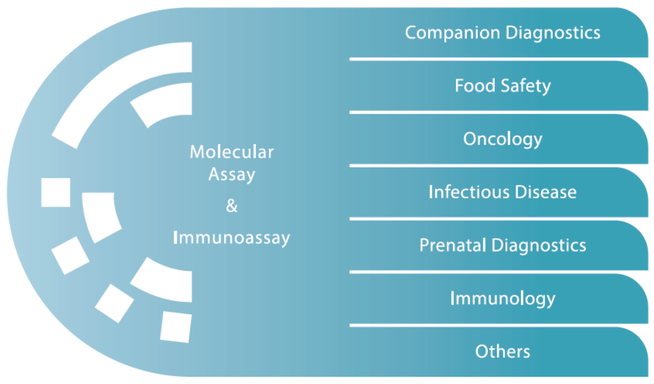



**Figure 3. Variations (CV%) in coupling efficiencies between 4 distinctly different DNA probes were determined using 3 lots of πCode™ MicroDiscs.**




## Applications




## Results

**Figure 4. Comparison of Mutation-Specific Signal between Healthy and Lung Cancer Patients** The signals for EGFR mutations are significantly higher in the cfDNA of lung cancer patients than those in healthy counterpart.

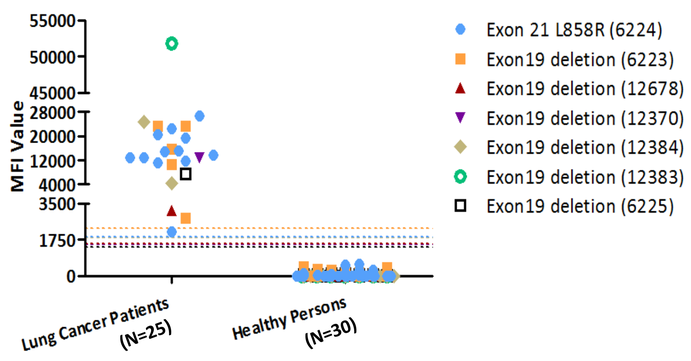



**Table 1. IntelliPlex™ Lung Cancer Panel for Cytology and Liquid Biopsy-** NSCLC mutations profiling determined by IntelliPlex™ Lung Cancer Panel is consistent with results tested by varies methods (RT-PCR, ddPCR) from third party site.

| Sample ID | Sample Type | Lung Cancer Panel (DNA) | | | | | External 3rd Party Single Gene Testing* |
|---|---|---|---|---|---|---|---|
| | | EGFR | KRAS | NRAS | PIK3CA | BRAF | |
| 1 | Cytology | T790M | WT | WT | E545Q | WT | EGFR L747_751>P & T790M |
| 2 | | WT | G12D | WT | WT | WT | EGFR WT |
| 3 | | WT | G12C | WT | E542K | WT | EGFR WT |
| 4 | cfDNA | p.L747_P753>S, T790M | WT | WT | WT | WT | EGFR L747_P753>S & T790M |
| 5 | | p.E746_A750del | WT | WT | WT | WT | EGFR E746_A750del & T790M |
| 6 | | p.L747_T751del | WT | WT | WT | WT | EGFR L747_T751del |
| 7 | | WT | WT | WT | WT | WT | EGFR WT |
| 8 | | WT | WT | WT | WT | WT | EGFR WT |

*Results verified by ddPCR for EGFR only

**Table 2. Comparison chart of IntelliPlex™ HCV Genotyping Kit results utilizing either plasma or serum derived form 63 specimens.**

| Genotype | Total Number of Samples | IntelliPlex Results (Serum) | IntelliPlex Results (Plasma) | Percent Correlation Serum/Plasma |
|---|---|---|---|---|
| G1 | 21 | 21 | 21 | 100% |
| G1a | 1 | 1 | 1 | 100% |
| G1b | 20 | 20 | 20 | 100% |
| G2 | 6 | 6 | 6 | 100% |
| G3 | 2 | 2 | 2 | 100% |
| G4 | NT | NT | NT | - |
| G5 | NT | NT | NT | - |
| G6 | 3 | 3 | 3 | 100% |
| G1b+G6 | 1 | 1 | 1 | 100% |
| Negative | 30 | 30 | 30 | 100% |
| **Total** | **63** | **63** | **63** | **100%** |

**Table 3. Comparison between Commercial ELISA- ToRCH Plus B19 Testing using Clinical Specimens**

| Targets | Status (Positive or Negative) | IntelliPlex* | Commercial Kits** |
|---|---|---|---|
| Toxoplasma IgG | POS | 7 | 7 |
| | NEG | 2 | 2 |
| Rubella IgG | POS | 7 | 7 |
| | NEG | 4 | 4 |
| CMV IgG | POS | 6 | 6 |
| | NEG | 3 | 3 |
| HSV1 IgG | POS | 8 | 8 |
| | NEG | 3 | 3 |
| HSV2 IgG | POS | 4 | 2 |
| | NEG | 7 | 9 |
| B19 IgG | POS | 9 | 9 |
| | NEG | 3 | 3 |

* Yields results for 6 targets from a single MWP well reaction
** Each target was analyzed by a commercial single target ELISA kit respectively

## Summary

- πCode™ Technology is a proven multiplexing platform for ready-to-use applications, capable of delivering both molecular & immunoassay diagnostic results.
- πCode™ Technology is designed for users to develop their own assays in a vast and wide range of applications.

## References

1. Gillian Ellison (2013), J Clin Pathol. 2013 Feb; 66(2): 79–89.
2. V.A. Morozov, S. Lagaye, Hepatitis C virus: Morphogenesis, infection and therapy., World J. Hepatol. 10 (2018) 186–212. doi:10.4254/wjh.v10.i2.186.

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