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ANALYTICAL PERFORMANCE OF ICE*Plex*™: AN AUTOMATED PLATFORM FOR SIMULTANEOUS DETECTION AND QUANTIFICATION OF MULTIPLE DNA TARGETS

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Primera Dx
Multiply Your Results

ICE*Plex*

Operational Features

- Integrated PCR/CE System
- Multiplex PCR capability
- Qualitative & Quantitative results
- Flexible run size
- Fully Automated
- Bench top system
- Automated workflow
  (minimum technician involvement)
- Run time of less then 5 hours

Ref: Garcia, E.P., et al. (2005) J. Molec. Diagn. 7(4):444-454

ICE*Plex* Simultaneously Detects & Quantifies Multiple Targets from PCR

Simultaneous, real-time sampling of PCR reaction mixtures by electrokinetic injection into CE capillaries during cycles 19 to 41 (alternate cycles only). Electropherograms (E-grams) were aligned, peaks identified by size (ID) relative to internal standards, peak areas were measured, and amplification curves constructed from these data. Quantification (QT) of analyte amplicons was derived by comparison to internal calibrators within every sample.

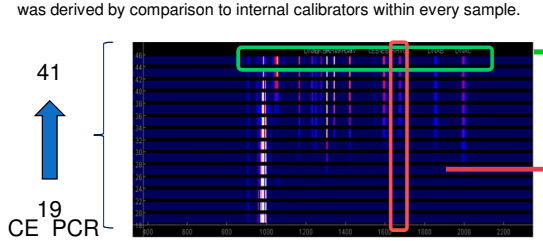
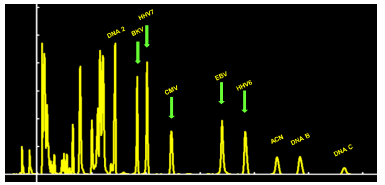
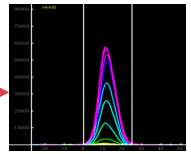


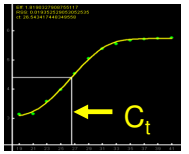
Image of fluorescent output compiled from 12 separations from a single well

E-gram from single well, last cycle





Expanded amplicon peak area over 12 cycles

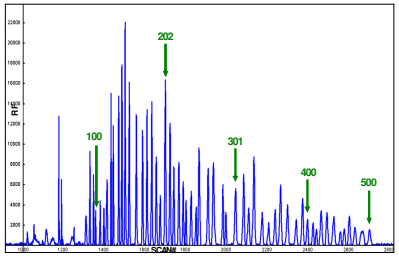


Corresponding derived C_t.

1 - Amplicon Size Detection Range

The Multiplex Ligation-dependent Probe Amplification (MLPA) kit for breast cancer markers (MRC-Holland, the Netherlands) was used to generate 52 amplicons with a single color label (FAM) between 100 and 500bp in size.

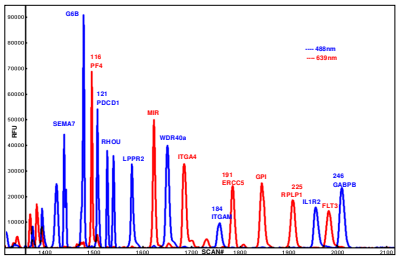
The ICE*Plex* instrument was able to separate and resolve all 52 peaks, demonstrating the capability for multiplexing with well over several dozen targets.



2 - Two-Color Capability

A 16-target multiplex assay was derived from the AlloMap gene expression test for assessing the risk of transplant rejection (XDx, Brisbane, CA). Two different colors were used, with 9 amplicons being labeled with FAM (blue) primers and 7 amplicons labeled with TYE (Cy5 analogue, IDT, Coralville, IA - red) primers. Detection of both colors was simultaneous and independent within each CE separation.

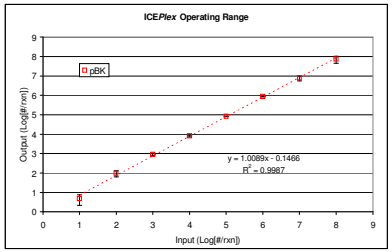
The use of 2 colors shows that the multiplexing capabilities of the ICE*Plex* system can permit an increased number of analytes detected, and they offer flexibility in designing target amplicon sizes.



3 - Target Operating Range

A cloned target sequence from BK virus was serially diluted from 10^8 down to 10 copies per reaction and run in triplicate in a prototype viral assay, in the presence of internal calibration controls.

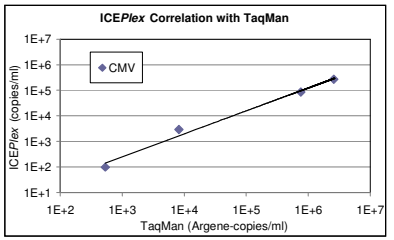
The ICE*Plex* detected this target DNA over a range of 7 orders of magnitude, demonstrating broad utility for detecting variable target concentrations.



4 - Correlation with TaqMan®

CMV viral particles were spiked into negative human plasma, extracted using the NucliSENS easyMAG system (bioMérieux), and the eluates run on the ICE*Plex* (prototype viral assay), or ABI 7500 system (Argene assay for CMV).

Results from the ICE*Plex* system correlated well with the TaqMan assay. The ICE*Plex* has the added benefit of multiplexed output from a single reaction, including extraction controls, internal calibrators, and potentially other analytes.



Log-Log Linear Regression		r2
CMV	y=0.899x + -0.298	0.988

Conclusions

1. High multiplexing capability
2. Flexibility in target amplicon selection and design
3. Broad dynamic range
4. Quality of multiplex quantification is comparable to singleplex TaqMan.

ICE*Plex* is For Research Use Only
Not for use in diagnostic procedures.