

H-1214: Performance Characteristics and Validation of the PhenoSense® Integrase Assay

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BACKGROUND:

- Raltegravir (RAL) was approved in late 2007 and is the first integrase strand transfer inhibitor in this new class of anti-retrovirals
- Several integrase inhibitors are in clinical development
- The PhenoSense® HIV Integrase Assay is a rapid, recombinant virus assay capable of measuring the susceptibility of HIV-1 integrase inhibitors, such as RAL, and integrase (IN) associated changes in replication capacity (RC).
- Here we report on the technical validation of the PhenoSense® HIV Integrase Assay in compliance with CLIA (Clinical Laboratories Improvement Act) specifications.

METHODS:

- The PhenoSense PR-RT assay was modified to capture patient-derived C-terminal RT and IN pol gene sequences (Figure 1).
- IN RC is expressed as a percentage of viral infectivity (luciferase production) relative to a reference virus.
- Assay accuracy, precision, reproducibility, linearity, amplification sensitivity and specificity were assessed by testing RAL susceptibility using site directed mutant (SDM) viruses, well-characterized laboratory strains, and patient plasma-derived viruses.

RESULTS:

- 100% of IC₅₀ FC values, for selected IN SDMs, were within 3 fold of previously reported phenotypic data (Table 1, Figure 2).
- 100% of pair-wise FC comparisons were within 2-fold for precision, reproducibility and linearity experiments (Table 1).

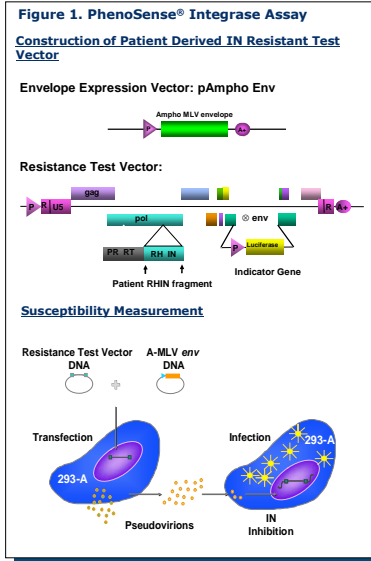


Table 1: Assay Performance: Accuracy, Precision, Reproducibility and Linearity Results

Acceptance Criteria:
 Phenotypic Susceptibility: >50% of pair-wise IC₅₀ FC comparisons must be within 2 fold
 IN RC: >90% paired comparisons must be within a +/-0.25 tolerance for log₁₀ IN RC

Accuracy	RAL IC ₅₀ FC	R10* IC ₅₀ FC	IN RC
% Acceptable	100%	100%	na
Total Comparisons	15	15	na
# Acceptable	15	15	na
Precision			
% Acceptable	100%	100%	94%
Total Comparisons	126	126	93
# Acceptable	126	126	87
Reproducibility			
% Acceptable	100%	100%	97%
Total Comparisons	103	103	97
# Acceptable	103	103	94
Linearity			
% Acceptable	100%	100%	98%
Total Comparisons	264	264	264
# Acceptable	264	264	258

*R10 is the raphyridine carbonyl L87P810 (Merck)

Figure 2. Comparison of PhenoSense® Integrase with Published Phenotypic Data

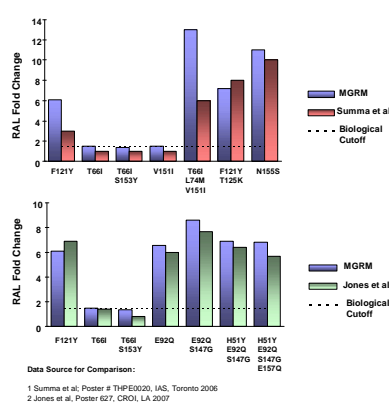


Figure 3. Amplification Sensitivity was Determined to be >500 copies/ml

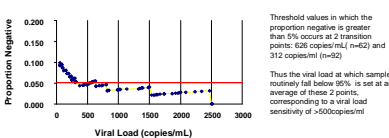


Table 2: Summary of Non Subtype B IN samples successfully amplified and phenotyped

Subtype	# Tested	# Successful	% Positive
A/A1	10	10	100%
AE	5	4*	80%
AG	5	5	100%
BF/F	5	4**	80%
C	15	15	100%
D	10	10	100%
G	5	5	100%
TOTAL	65	63	96.4%

* All 5 samples amplified but 1 out of 5 was non-functional
 ** 1 sample failed amplification

Figure 4. Distribution of RAL IC50 Fold Change Values in Integrase Inhibitor Naïve Patient Samples

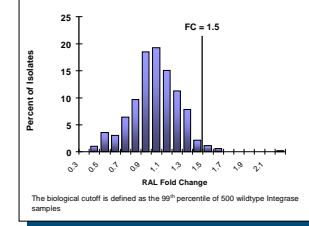


Figure 5. Susceptibility of Commercial Samples Tested using PhenoSense® Integrase

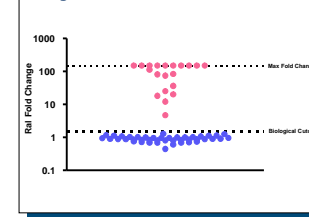


Figure 6. PhenoSense® Integrase Assay Report



RESULTS (cont.):

- For IN RC, 94%, 97% and 98% of paired comparisons were within +/-0.25 log₁₀ for precision, reproducibility and linearity, respectively (Table 1).
- Amplification sensitivity was successful for 95% of samples with viral loads above 500 copies/mL (Figure 3).
- Sensitivity to amplify and phenotype Non Subtype B IN sequences was 96.4%, as determined from a panel of diverse Non Subtype B isolates (A/A1,AE,AG,BF/F,C,D,G) (Table 2).
- The biological cutoff for RAL FC was determined to be 1.5 using 500 IN inhibitor naïve patient samples (Figure 4).
- In 63 commercial samples tested using PhenoSense® Integrase, RAL resistant and sensitive samples were identified (Figure 5).

CONCLUSIONS:

- PhenoSense® Integrase is an accurate, precise, reproducible assay for assessing IN inhibitor susceptibility and changes in IN RC associated with IN inhibitor resistance.
- This assay is performed in the Monogram Clinical Reference Laboratory on samples with viral loads above 500 copies/mL.
- PhenoSense® Integrase provides clinicians with a tool to aid in the selection and monitoring of potent antiretroviral combination therapy (Figure 6).

ACKNOWLEDGEMENTS

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