

# Immunoscore® Colon analytical performance

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## Background

The Immunoscore® was validated as a powerful prognostic marker in colon cancer in a study conducted by the Immunoscore® worldwide consortium, led by the Society for Immunotherapy of Cancer (SITC) involving 23 pathology centers from 17 countries, and including more than 3800 patients.

HaliuDx has developed a standardized version of the test that was used in this study. Here we show the concordance with the research version and present the main analytical performances of the system.

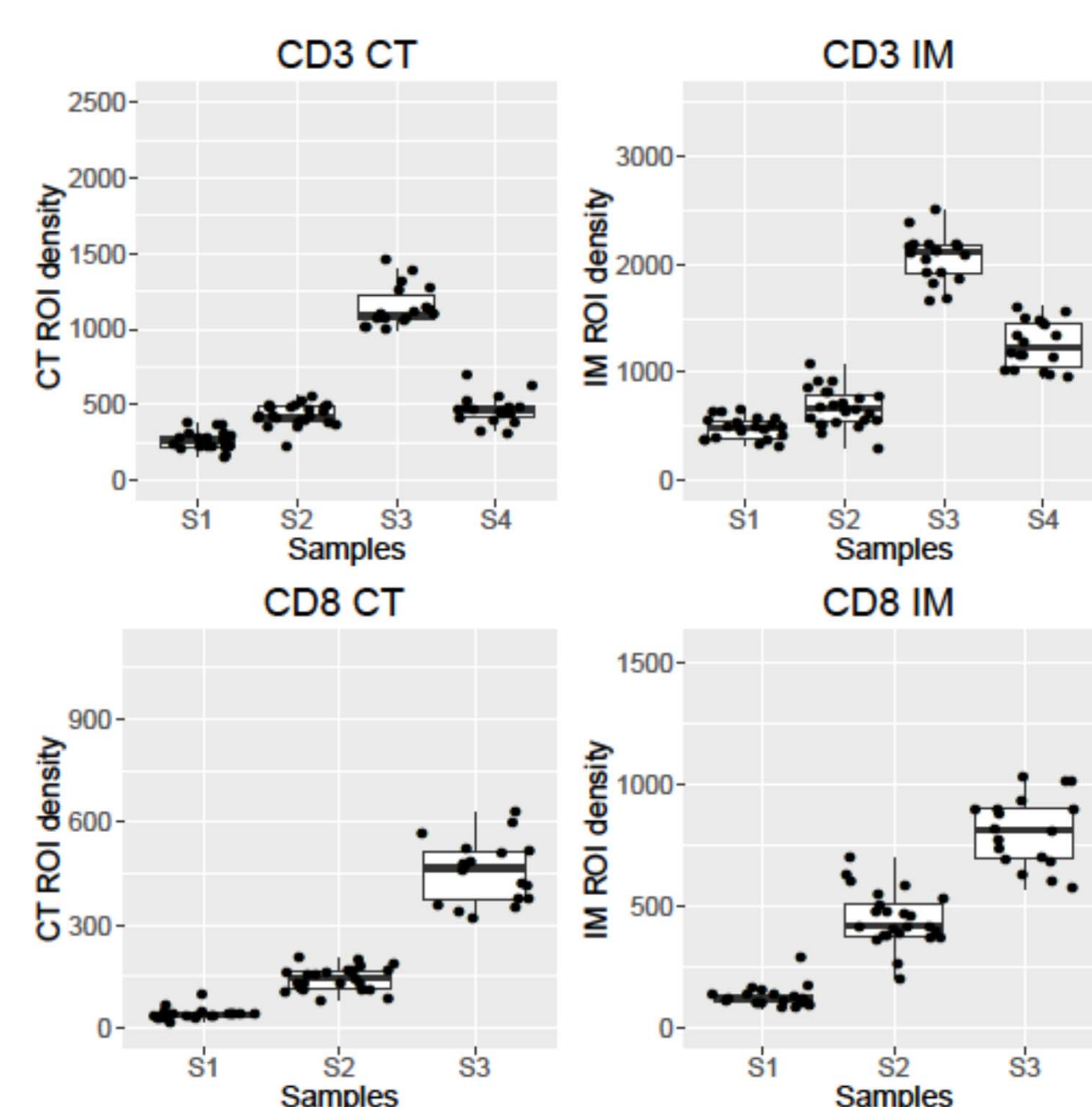
## Method

For each colon tumor block, 2 slides are stained using an automated IHC staining instrument: one with CD3 and one with CD8. Digital images of stained slides are obtained using a whole slide scanner, and analyzed by a software program (Immunoscore® Analyzer, HaliuDx). The Immunoscore® Analyzer automatically processes images for tissue detection (core of the tumor CT and invasive margin IM). Densities of positive lymphocytes in CT and IM are reported.

For each marker and each zone, densities distributions have been established during the SITC study. The Immunoscore® is reported in 5 categories from 0 to 4, or as Immunoscore® High (IS-3 and IS-4), Low (IS-0 and IS-1) & Intermediate (IS-2).

## Precision

- ✓ 4 FFPE Colon Cancer samples – 144 slides stained with CD3 and CD8 antibodies
  - 2 autostainers (Benchmark)
  - 3 revelation reagents lots
  - 3 Immunoscore® Colon kit lots
  - 6 IHC runs
  - 3 operators
- ✓ 42 slides for Sample 1 (22 CD3 and 20 CD8)
- ✓ 48 slides for Sample 2 (24 CD3 and 24 CD8)
- ✓ 36 slides for Sample 3 (18 CD3 and 18 CD8)
- ✓ 18 slides for Sample 4 (18 CD3)

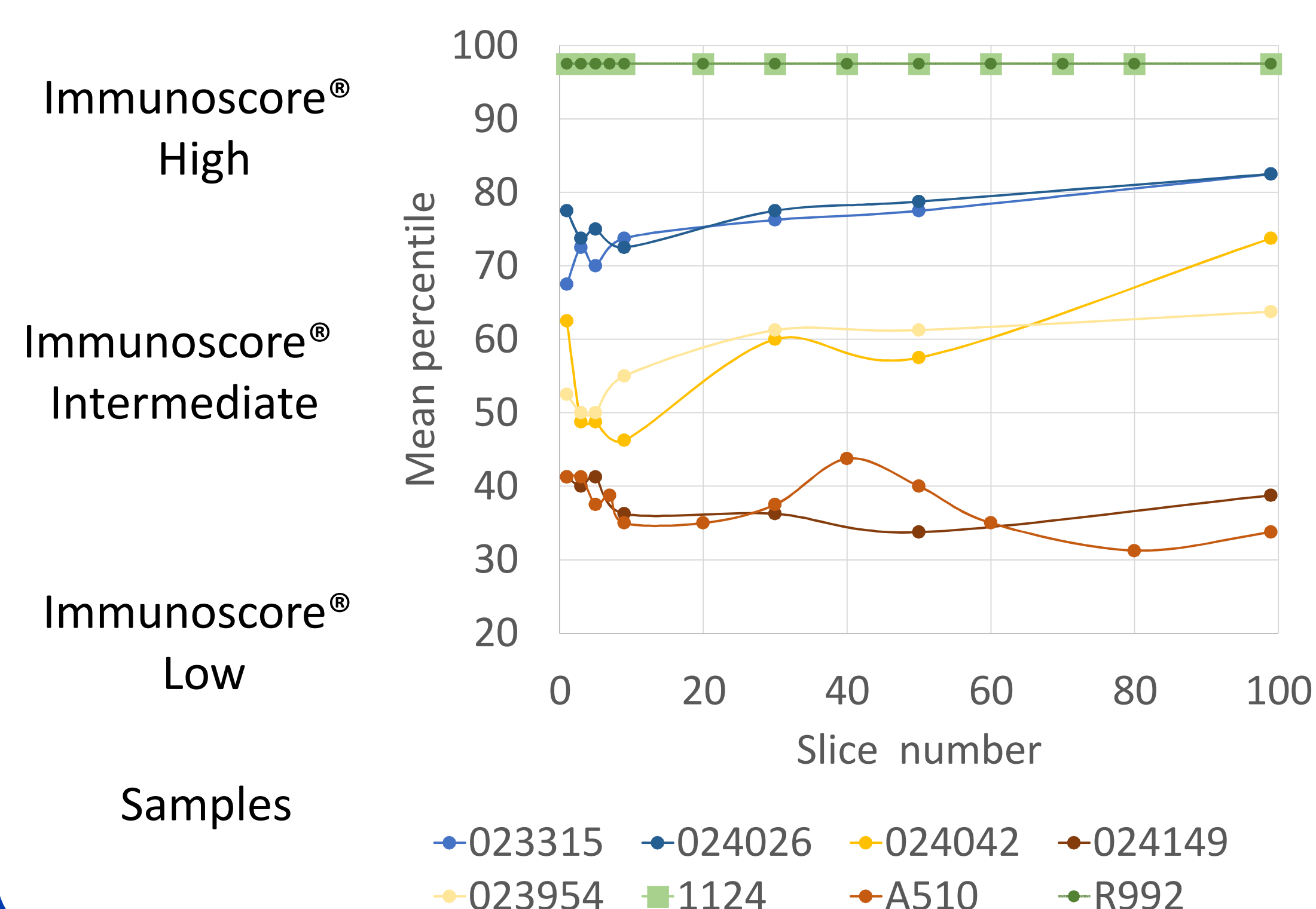


- ✓ CV inter-instrument: 0-12%
- ✓ CV inter-lot: 0-22%
- ✓ CV inter-operator: 0-18%

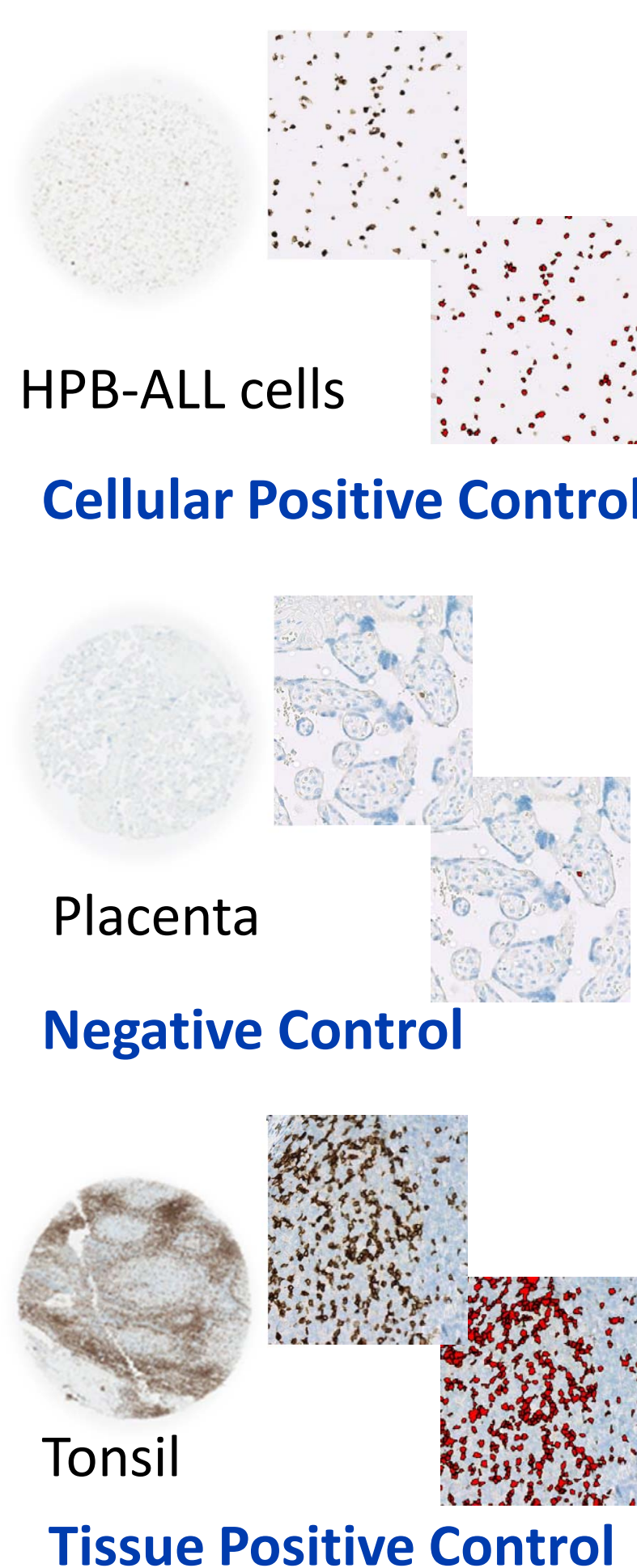
Sample	Low		Intermediate	High
	IS-0	IS-1	IS-2	IS-3
Sample 1	1	19	0	0
Sample 2	0	0	24	0
Sample 3	0	0	0	18

## Sample Heterogeneity

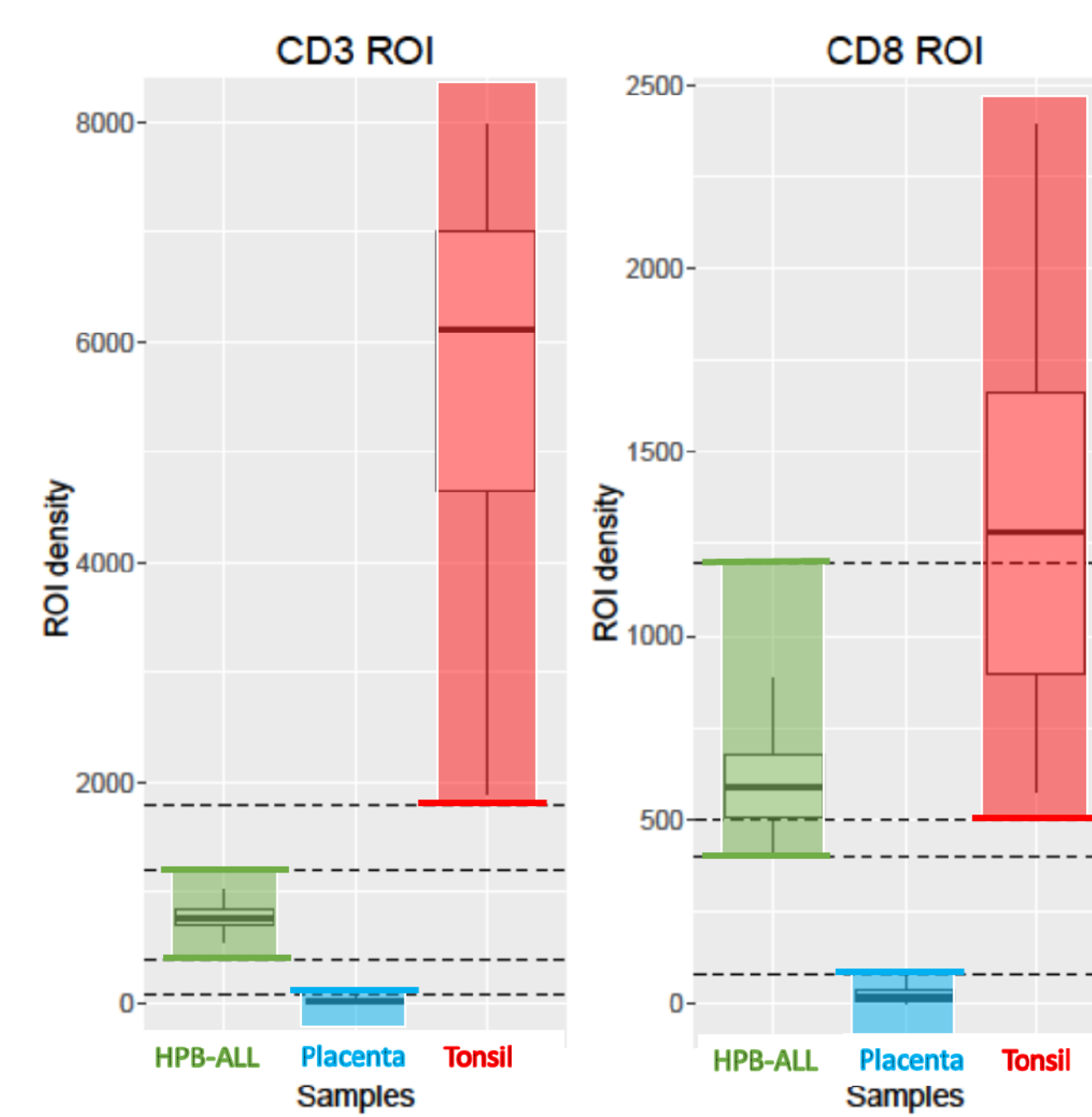
- ✓ 8 FFPE blocks were cut in 100 slices
- ✓ Immunoscore® was measured along the block showing very good homogeneity of the marker



## Controls



TMA slides containing 3 cores are processed in the same IHC run as Colon Cancer slides, providing quantitative run Quality Control



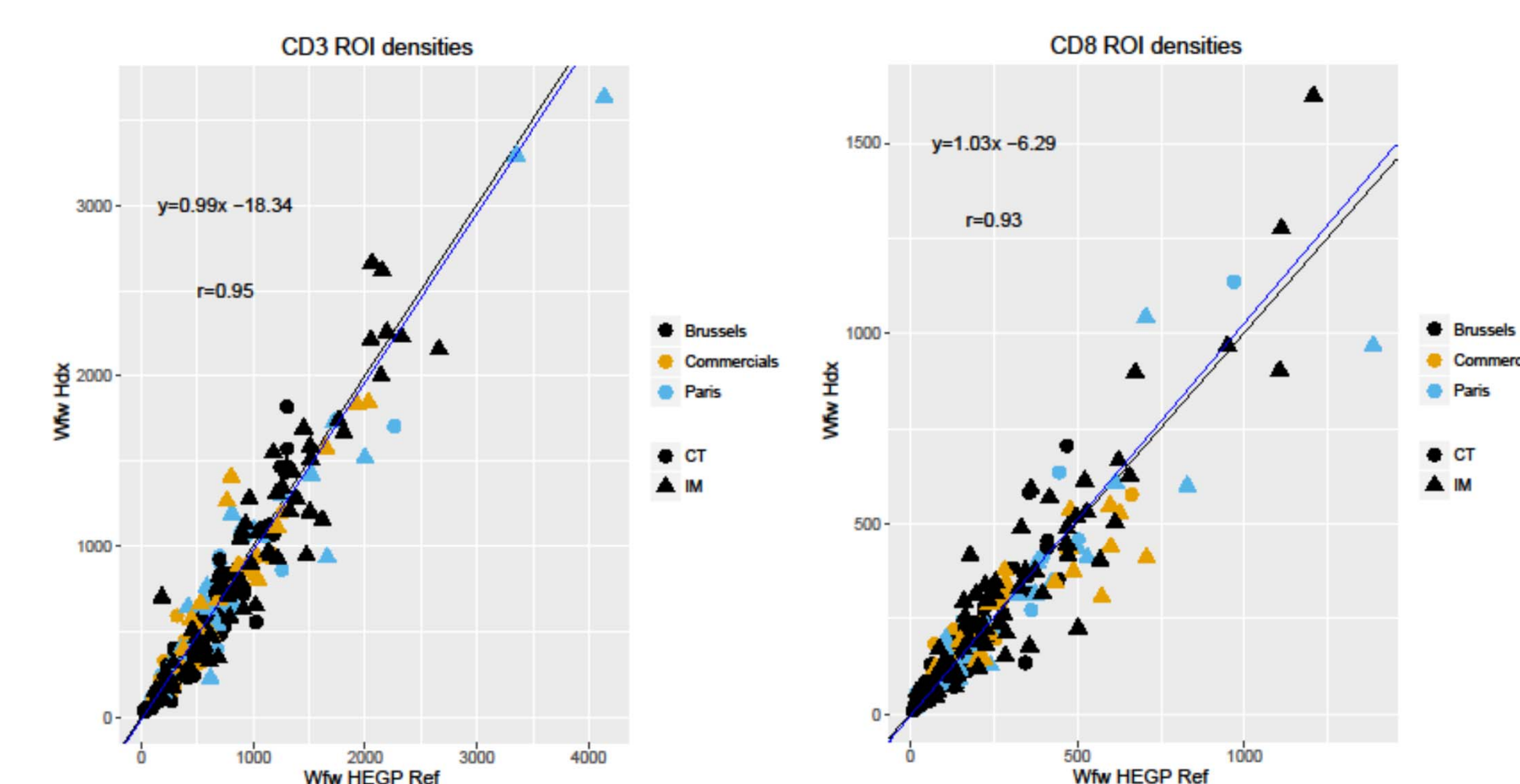
## Accuracy

Objective = compare the quantification results obtained using the SITC reference and the HaliuDx workflows (WF)

These 2 WFs differed in terms of:

- Antibody provider / clone / dilution

- Scanner instrument
  - Analysis software
- 115 samples from different sources were tested:
- Paris – HEGP
  - Brussels – Cliniques Universitaires Saint-Luc
  - 3 commercial suppliers



HaliuDx	Reference SITC study (HEGP - Paris)					
	Low	Intermediate		High		
		IS-0	IS-1	IS-2	IS-3	IS-4
Low	13	6	0	0	0	
Intermediate	3	15	6	0	0	
High	0	0	48	4	0	
	IS-3	0	0	2	15	0
	IS-4	0	0	0	0	2

**Table 1:** Concordance between HaliuDx and reference workflows. For the Immunoscore® in 3 groups, the overall agreement is 89% [ 82 - 93 ]  
Low x High agreement is 100% [ 93 - 100 ]

## Conclusions

The inter-instrument, inter-lot and inter-operator/ -reader precision in terms of cell density (cells/mm<sup>2</sup>) CV were below 12%, 22% and 18%, respectively. Only 1 change in Immunoscore® category (out of 62 IS assessments) was observed, from IS-1 to IS-0.

The equivalency between HaliuDx and SITC-recommended workflows was assessed in terms of cell densities. Deeming regression slopes were not significantly different from 1 for both CD3 and CD8 antibodies. Pearson correlation coefficients were above 0.93. The concordance table is provided in Table 1, corresponding to a weighted Cohen's kappa coefficient of 0.9.

The Immunoscore® Colon is a robust, easy-to-use and accurate assay. It is the first standardized immune-based assay for the classification of cancers.

## References

1. Galon J, et al. J Pathol 2014, 232(2):199-209.
2. Galon J, et al. J Clin Oncol 34, 2016 (suppl 3500).