

Proficiency Panels: ELISpot Consistency Between Labs 2021

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Collaboration Between Immudex, CIC/CRI, and CIMT



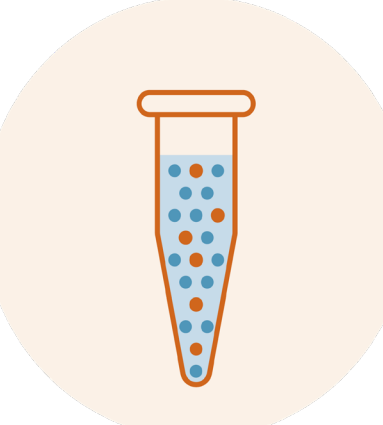


Immudex offers Proficiency Panels in collaboration with CIC (the US Cancer Immuno-therapy Consortium of the CRI) and CIMT (the European Association for Cancer Immunotherapy) to help researchers and clinicians worldwide evaluate their immune monitoring performance with the MHC Multimer and T-cell ELISpot assays. This poster focuses on the ELISpot Proficiency panel 2021.

Proficiency Panels provide:

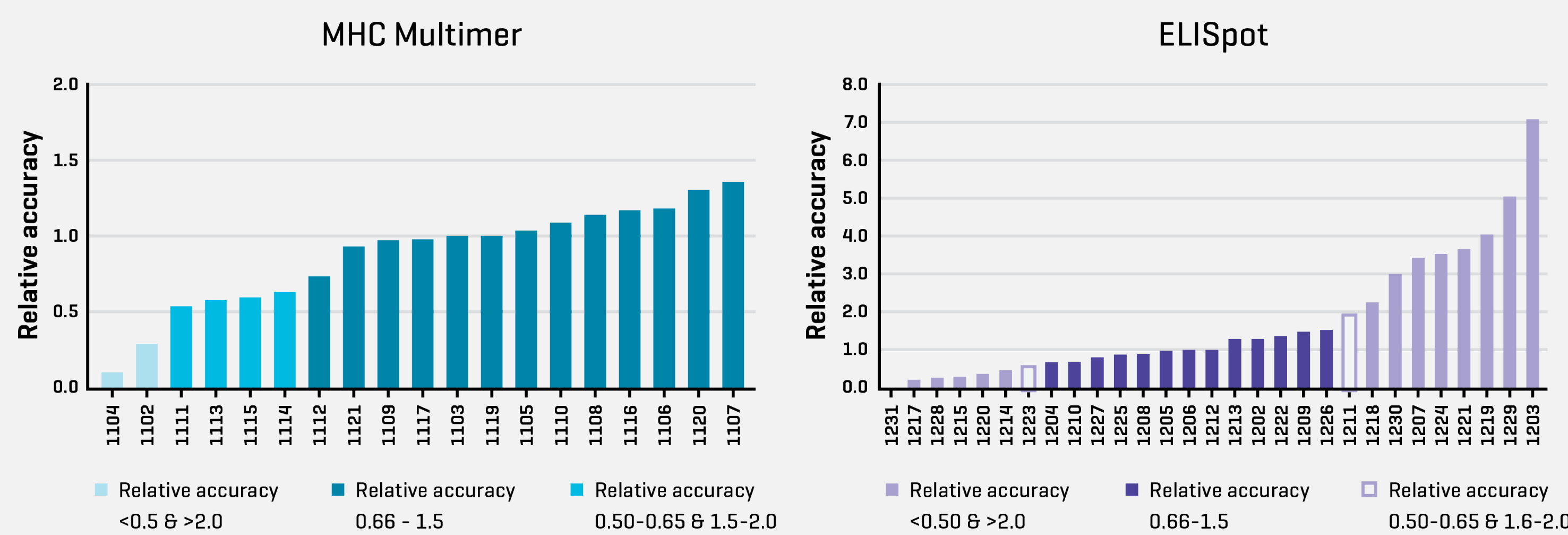
- External validation of assay performance
- Enhanced assay harmonization
- Coordinated guidelines for MHC multimer and T-cell ELISpot assays
- Proficiency panel reports

Participation is Easy!

A test cycle takes 4 months to report delivery and results are anonymous.

				
Sign up Quickly register to participate in a test cycle. Your name and affiliation are kept anonymous	Receive Samples All participants receive identical PBMC samples to analyze	Analyze Samples Use your lab-specific protocols to process the PBMC samples according to instructions	Upload Data Report your results back to Immudex	Receive Report Receive a full report on the test cycle with anonymized performance of all participants

MHC Multimer Results are Most Consistent Between Different Laboratories



When comparing ELISpot to MHC multimer technology such as Dextramer® reagents in the Proficiency Panels performed in 2020 by multiple different laboratories, MHC multimers were more consistent and reproducible.

- ELISpot Proficiency Panel:** participants determine the number of IFN-γ secreting antigen-specific T cells in CMV-positive human PBMC samples
 - Results: 13 of the 29 participants (44.8%) had a relative accuracy between 0.66-1.5 and were considered “in the average range” (dark purple columns).
- MHC Multimer Proficiency Panel:** participants determine the amount of EBV-specific T-cells in a EBV-positive sample using MHC and Negative Control MHC Multimers.
 - Results: 13 out of 19 participants (68.4%) had a relative accuracy between 0.66 – 1.5 and were considered “the average range” (dark blue columns).

ELISpot Proficiency Panels 2021

In the T-cell ELISpot Proficiency Panel 2021, 29 participants from 11 countries reported their data. 22 participants were from Academia, and 7 participants were from industry. The participants measured the number of IFN-γ secreting antigen-specific T cells in two different PBMC samples (PBMC 2010113745 and HHU20180918 stimulated with CMV and CEFX peptide pools. In advance, the PBMCs were pre-tested by the external partner Mabtech AB (Sweden).

Data analysis no.	PBMC	Reagent	Pre-test result
1	HHU20180918	Reagent 1 (CMV) and Reagent 3 (Negative Control)	Low response
2	HHU20180918	Reagent 2 (CEFX) and Reagent 3 (Negative Control)	Medium/high response
3	2010113745	Reagent 2 (CEFX) and Reagent 3 (Negative Control)	Medium response
4	All	Overall Proficiency score	-

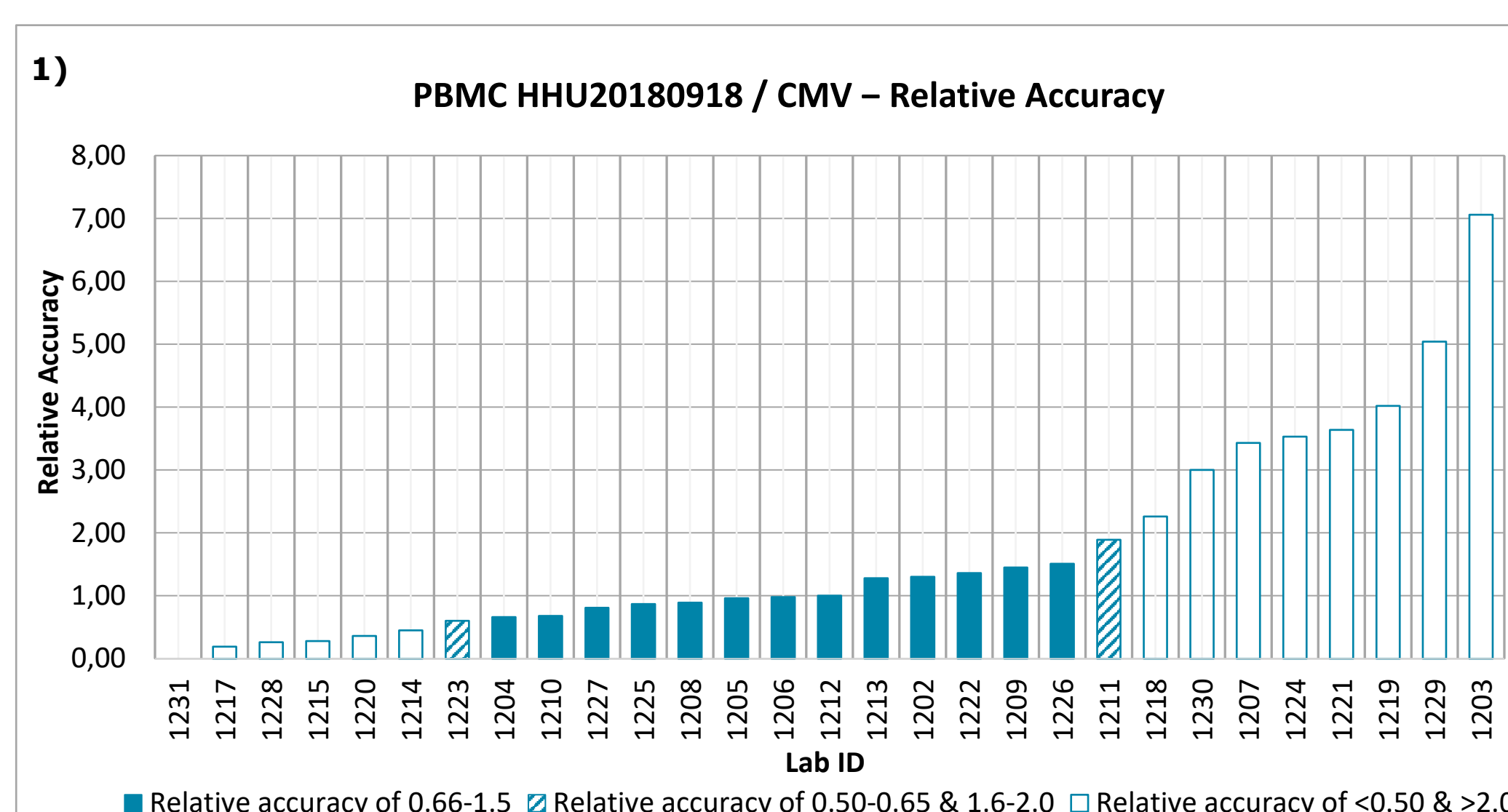
Results

The following variations in protocols are seen between laboratories:

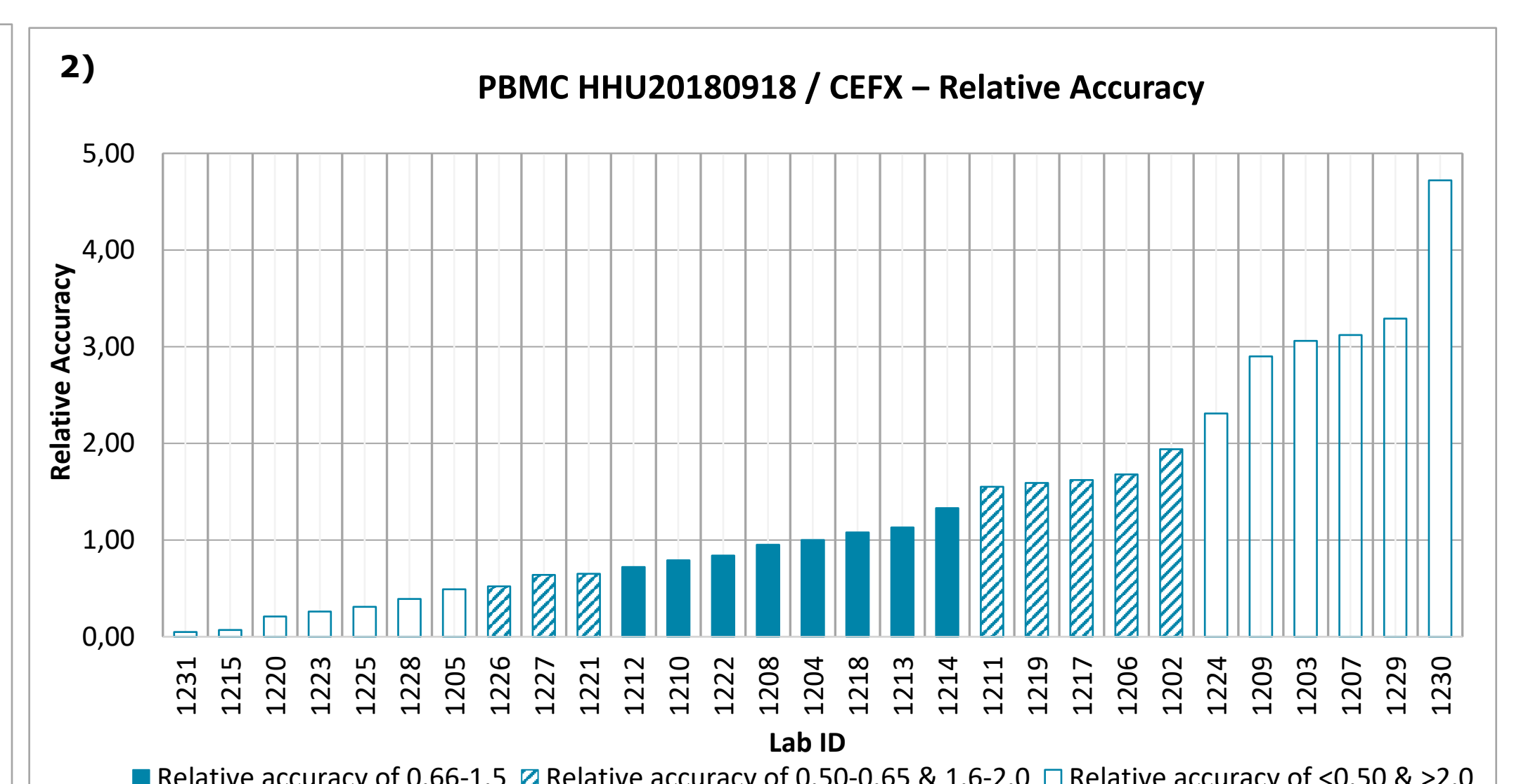
- High performing serum/medium
- Overnight resting
- Assessment of apoptotic cells

Overall results

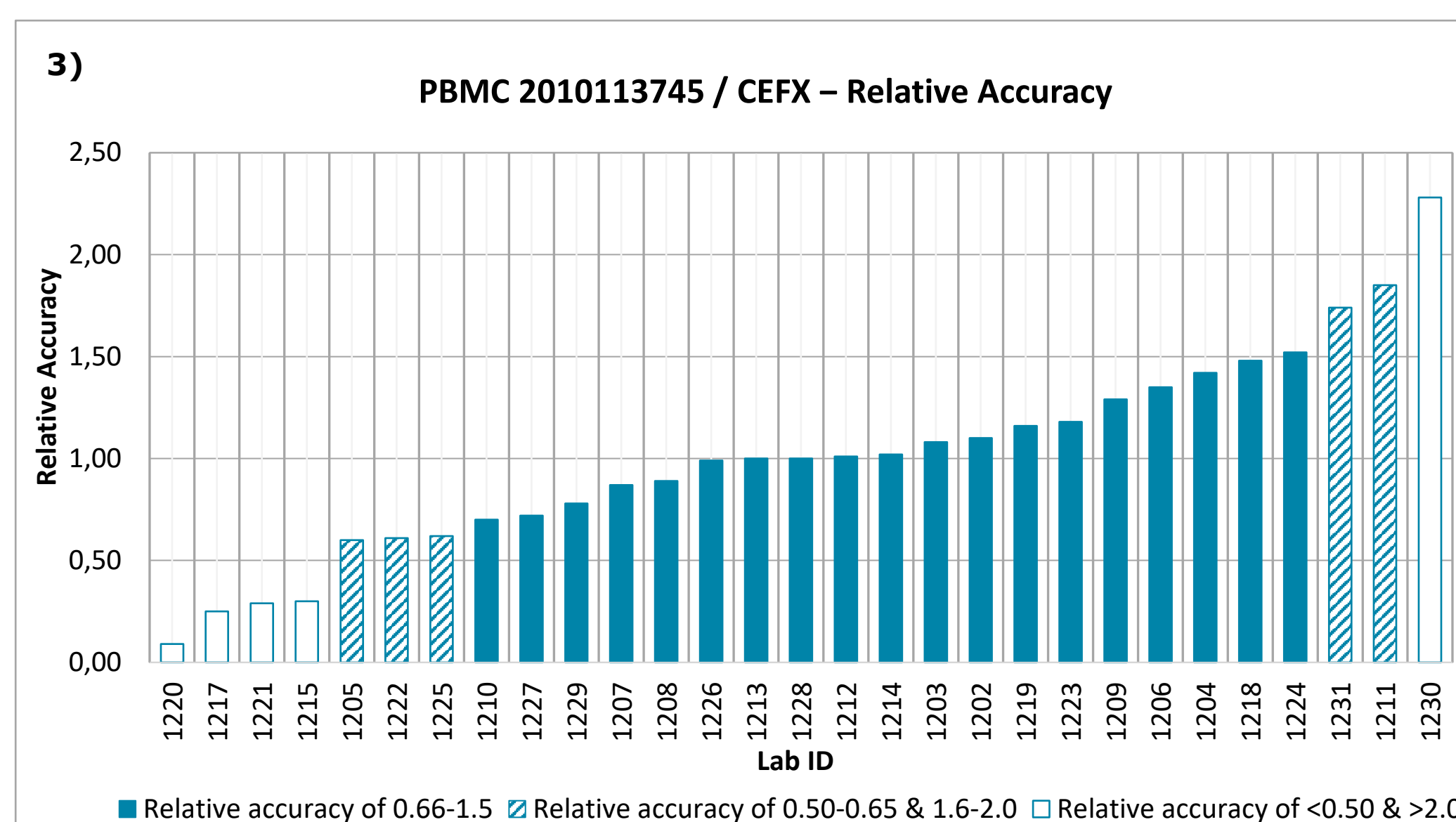
- PBMC 2010113745 was found to be negative for CMV and positive for CEFX, and PBMC HHU20180918 was positive for CMV and CEFX.
- The two samples with lower frequency of antigen-specific T cells (1, 2), results were less aligned than the sample with higher frequency of antigen-specific T cells (3).
- The sample with the highest panel median (3) was the one where most participants (19 out of 29) obtained results within the average range.
- Overall, 63% of the participating laboratories got a proficiency score of ≥ 2.0 (4). All measurements were made in triplicates, and here presented as mean values.



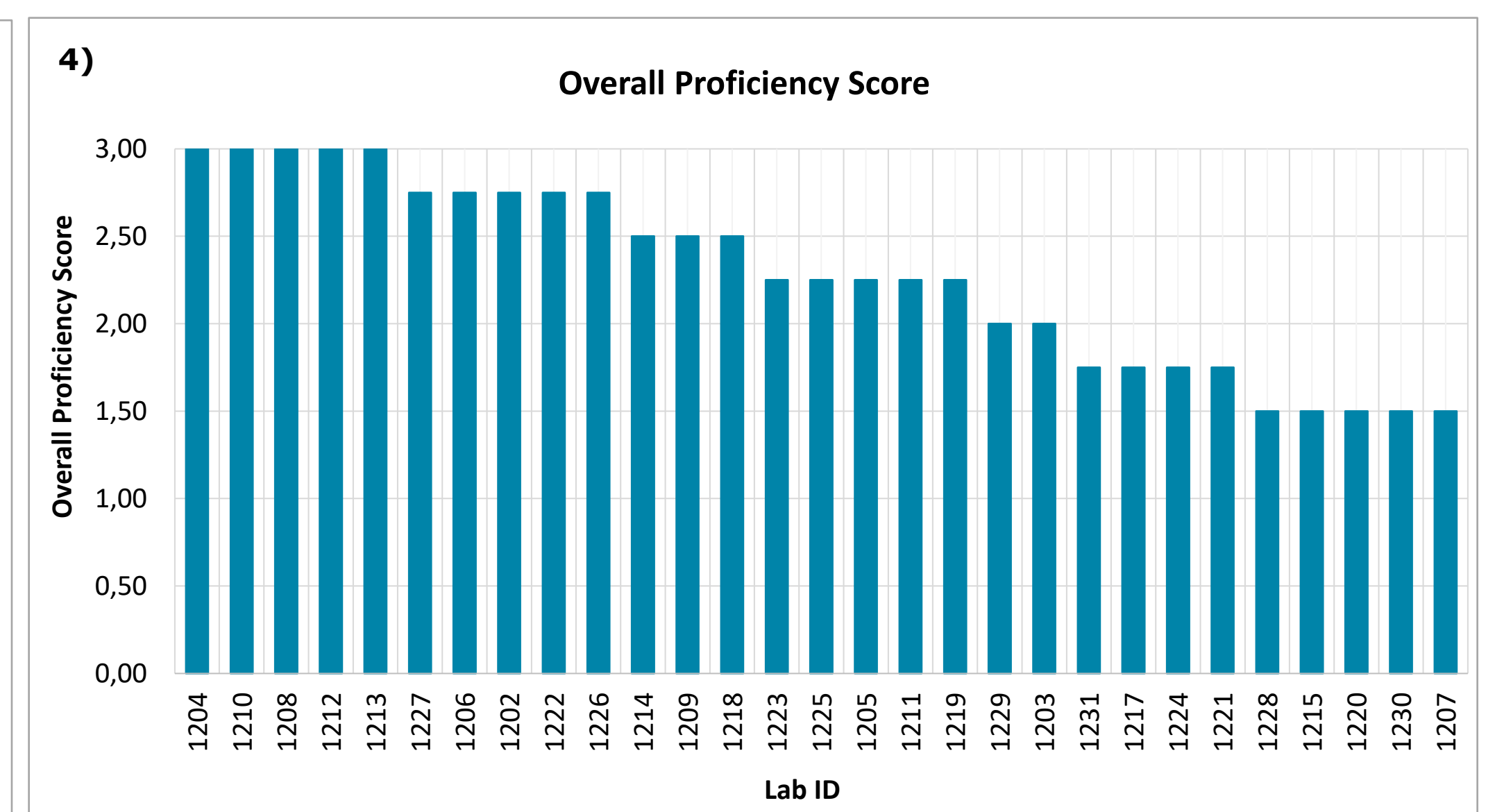
Relative accuracy for analysis of PBMC HHU20180918 with Reagent 1 (CMV). 13 of the 29 participants had a relative accuracy between 0.66-1.5 and are therefore considered "in the average range"



Relative accuracy for analysis of PBMC HHU20180918 with Reagent 2 (CEFX). 8 of the 29 participants had a relative accuracy between 0.66-1.5 and are therefore considered "in the average range"



Relative accuracy for analysis of PBMC 2010113745 with Reagent 2 (CEFX). 19 of the 29 participants had a relative accuracy between 0.66-1.5 and are therefore considered "in the average range"



Overall Proficiency Score in the T-cell ELISpot Proficiency Panel 2021.

ELISpot Proficiency Panel results. Graphs show relative accuracy of triplicates.

Relative Accuracy = $\frac{\text{Mean}}{\text{Median}}$

Relative accuracy	Corresponds to
0.66 – 1.5	within the average range
0.50 – 0.65	near the average range
1.6 – 2.0	far from the average range
< 0.50	
> 2.0	

Conclusions

- Similar triplicate results observed for PBMCs stimulated with both the CMV and CEFX peptide pools and the negative control
- T-cell ELISpot assays are more harmonized across different laboratories when looking at high-frequent T-cell responses than low-frequent responses
- 63% of the participating laboratories got a proficiency score of ≥ 2.0 .
- Proficiency Panels are a useful tool to evaluate the proficiency of immune monitoring assays across different laboratories to ensure comparable results in e.g., multicenter trials.

The full ELISpot Proficiency Panel 2021 report is available at www.immudex.com