Modernisation of Immunohistochemistry: Standardised Controls and a Novel Nanoparticle Labelling System

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INTRODUCTION

Immunohistochemistry (IHC) became a major tool in the diagnostic histopathology setting in recent decades. Today, there are a plethora of IHC markers that are highly selective for important diagnostic cellular components. There are also important companion diagnostic IHC assays that stratify cancer patients for response to targeted therapies. Since IHC became established as a clinically important part the pathology service across the world, there were no universally available reference standards for IHC testing. This is now changing. During 2021, a group of experts in the field of pathology and immunohistochemistry came together to form **the Consortium for Analytic Standardization in Immunohistochemistry (CASI)**. The mission of the expert CASI team was to create universally available validated reference standard controls for all the important biomarkers. These controls are known as calibrators. There are usually 4 calibrator spots that comprise of specific target antigens with a concentration range from low to high. These standardised **controls represent a significant improvement on tissue section controls as they do not suffer from variation in section thickness and variable antigen expression due to the nature of the disease, or uneven staining due to uneven fixation.** Whilst CASI were working on the production of robust standardised controls for IHC, a team with expertise in **Nanotechnology at Lumito in Sweden,** were working on a **novel labelling system** for IHC. Their novel nanoparticle labelling has the potential of replacing Diaminobenzidine/peroxidase labelling for a number of important companion diagnostic tests as it promises to provide a greater lower limit of detection. Also, this technology may offer the possibility of an accurate count of target antigen/epitopes within the companion setting. If so, this could be a significant advance when looking for low level expression within assays, such as those for HER-2 and PD-L1.

THE STUDY RECENTLY PUBLISHED BY CASI

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By employing standardized calibrators covering the full spectrum of HER-2 expression, CASI carried out an extensive study on commercially available assays using multiple microtissue arrays of FFPE breast cancers. The TMA's included a range of HER2 that were scored from 0 to 3+. The study was recently published by Dabbs et al.(see below) showed that the established HER2 IHC assays for identifying HER2 overexpression did not have the dynamic range to reliably demonstrate low HER2 expression. HER2 IHC assays with a higher analytic sensitivity, combined with HER2 image analysis, overcame the limitation. Figures 1-3 illustrate key aspects of the processes employed, whilst figure 4 provides an overview of each assay performance within the study.

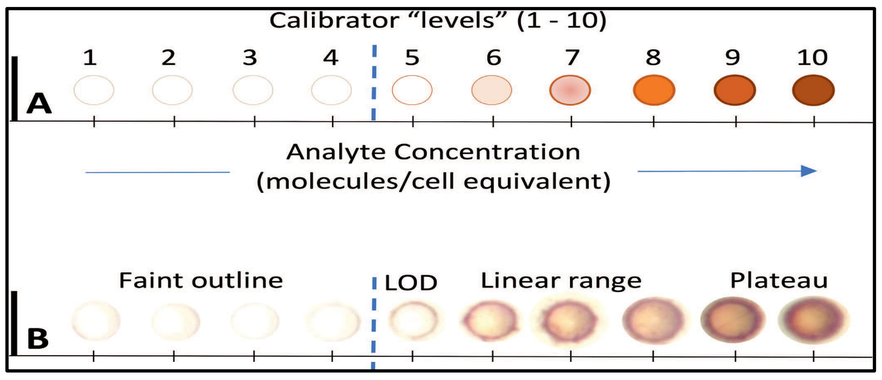


Figure 1. Illustration of a series of immunohistochemistry calibrators – as seen under the microscope - after staining. The numbers refer to calibrator levels, from low (1) to high (10) analyte concentrations. A, The illustration shows that rim staining is stronger than central staining because the analyte is attached to the microbead surface. In this example, level 5 represents the lower limit of detection (LOD). B, Images of microbeads from calibrators with an LOD at level 5. A Certificate of Analysis reveals the exact analyte concentration for each level.

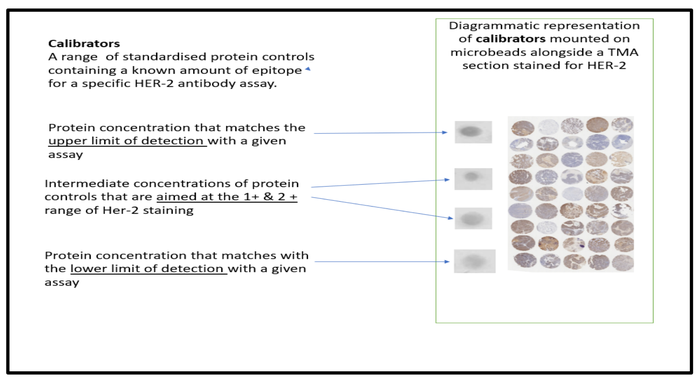


Figure 2. Illustration of TMA's of FFPE breast cancers with a range of HER-2 expression on slides with 4 protein calibrators alongside.

Figure 3. Roche DP 200 scanner employed to scan the TMA's so that the HER-2 expression could be assessed using Visiopharm and ImstarDx digital analysis. The TMA slides were also assessed manually by appropriately qualified histopathologists

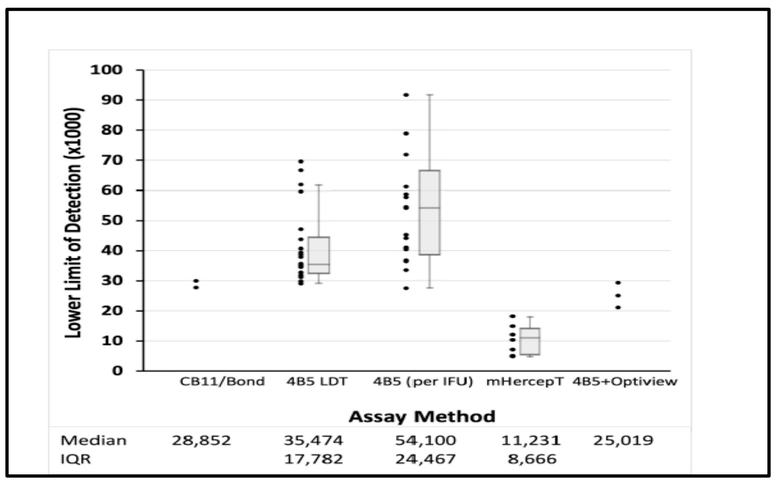
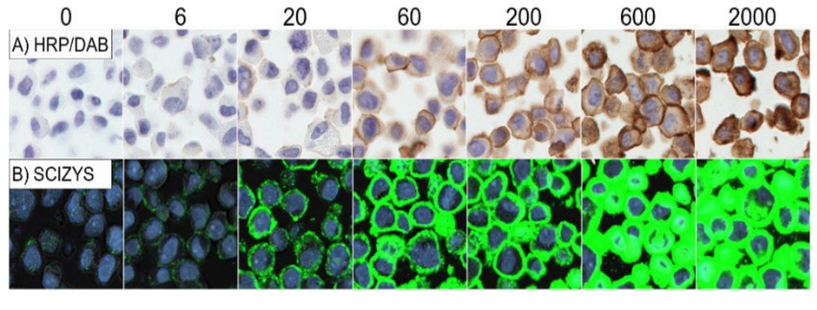


Figure 4. Analytic sensitivity, as measured by the lower limit of detection (y axis), for the various commercial assays used by participating laboratories (x axis). Each dot is a separate laboratory. A box plot is also illustrated with each data set showing the median, 25th and 75th quartiles, calculated exclusive of the median because each group has an even number of samples. Assay nomenclature: "4B5 (per IFU)" is the Roche PATHWAY assay without modification; "4B5 LDT" is the same reagents and instrument but after modification of the protocol, often extending an incubation time; "4B5+Optiview" is the same but coupling the primary antibody to the Optiview detection system; "mHercepT" is the Agilent DG44 monoclonal HercepTest on the Dako Omnis; "CB11/Bond" is the Leica Biosystems CB11pri-mary antibody pre-dilute on a Leica immunostainer. Lower limit of detection (y axis) is in ERF units per cell equivalent (Methods) and is intended to be multiplied ×1000.

Novel Nanoparticle Labelling for Immunohistochemistry: SCIZYS Developed by Lumito, Sweden

SCIZYS Erbium-SA is a new label type based on photon-upconversion nanoparticles (UCNPs). UCNPs are near-infrared (NIR) absorbing luminescent nanoparticles with an excitation maximum (976 nm) in the NIR optical window of tissue.[1] Unlike conventional fluorophores emitting light with lower energy/longer wavelength upon excitation (Stokes shift), UCNPs absorb more than one photon per excitation process and emit photons with higher energy/shorter wavelength (anti-Stokes shift).[2] This photon upconversion process completely removes tissue autofluorescence. It greatly enhances the detection sensitivity of the system, enabling the visualisation of individual UCNPs. In addition, UCNPs possess extreme photostability and can thus be handled under ambient light and maintain a constant emission over hundreds of scan cycles.Here, we show (figure 5) some of the outstanding properties of UCNP-based immunohistochemistry (IHC) labels in the SCIZYS system.



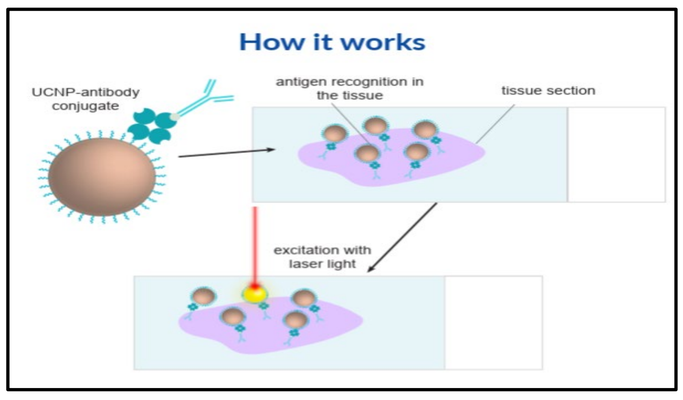


Figure 5. Staining workflow. Standard IHC workflow can be used with UCNPs. UCNP-antibody conjugates are formed and used to visualise antigens detected with standard primary antibodies. Fluorescent signal is obtained via UCNP excitation with a laser.

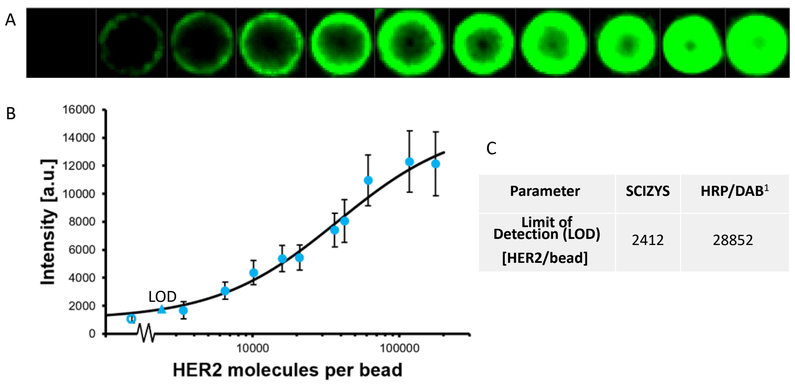


Figure 6. A comparative study shows that the SCIZYS labelling has a lower limit of detection than HRP/DAB. A) Boston Cell Standards HER2 IHCalibrators® were labelled with UCNPs using antibody CB11. B) Intensities of UCNP labelled beads were measured, mean and standard deviations were calculated and plotted for each bead population. C) LOD calculated using SCIZYS UCNP as reporter and compared to DAB from literature[1].

Figure 7.

SCIZYS Assay Sensitivity

For assessing the assay sensitivity of the SCIZYS system, a HER2 primary antibody titration was performed and compared with the HRP/DAB gold standard on FFPE Sections.The polymeric HRP/DAB secondary antibody label showed faint brownish membranous labeling on BT474 cells at 20 ng/mL of primary anti-HER2 antibody and clear membranous labelling at 60 ng/mL of primary antibody.

SCIZYS Erbium-SA was bound to the primary antibody via a biotinylated secondary antibody. The assay showed noticeable membranous labelling at 6 ng/mL of primary antibody. Strong membranous labelling was observed at 20 ng/mL .

References

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