

Utility of Upconversion Nanoparticles (UCNP) Based Immunohistochemistry in Diagnostic Renal Biopsies - A Proof of Concept Study



Coventry and Warwickshire
Pathology Services

Kishore Gopalakrishnan¹, Yee Wah Tsang¹, Matthias J. Mickert²

1. University Hospital Coventry and Warwickshire (UHCW) NHS trust, Coventry, UK

2. Lumito AB, Lund, Sweden



Background:

A Renal biopsy is an important tool in the management of renal disease and its analysis involves immunohistochemistry (IHC). The current methods, immunofluorescence (IF) and immunoperoxidase (IP), have their limitations. IF requires fresh, unfixed tissue, and IP results are frequently affected by excessive background staining. In our study, we explored a new approach using Upconversion Nanoparticles (UCNP) labeling for IHC.

Aim:

To compare conventional renal immunoperoxidase based IgA immunohistochemistry with a novel IgA immunolabelling reagent kit based on upconversion of nanoparticles; and using a whole slidescanner allowing for digital imaging of the labelled samples.

Methods:

Five biopsies previously diagnosed as IgA nephropathy were identified from UHCW archives and unstained sections obtained from these. One section from each case was stained using conventional DAB based IP techniques and another with UCNP IHC. These stained slides were scanned on a slide scanner and a pathologist reviewed and compared both sets of images.



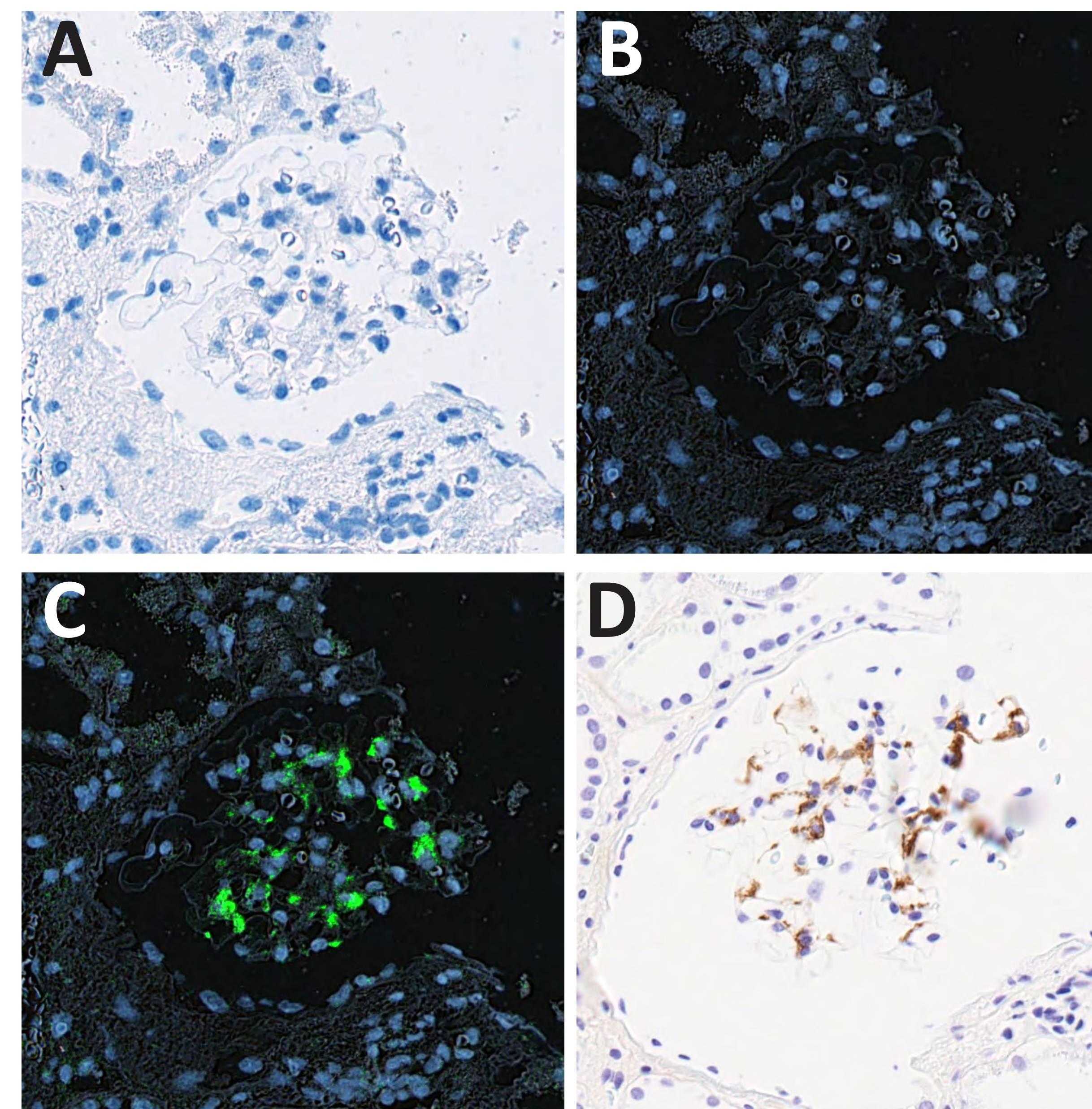
The SCIZYS S1 slide scanner was used. Upon inserting the slides, the scanner automatically takes overview images of the slides. The user can then select the scanning area for each slide.

The scanner first takes a brightfield image followed by the UCNP image at the exact same location (20x magnification). To acquire the UCNP image, the scanner excites the nanoparticles with near infrared light (980 nm), whereupon the UCNPs absorb two or more of the low-energy photons and convert these to a photon with higher energy (visible light). This anti-Stokes process drastically reduces the measurement background by eliminating tissue autofluorescence.

The digital whole slide images are saved and can be viewed in suitable platforms. For better visibility, the brightfield colours are inverted and the UCNP emission changed to green.

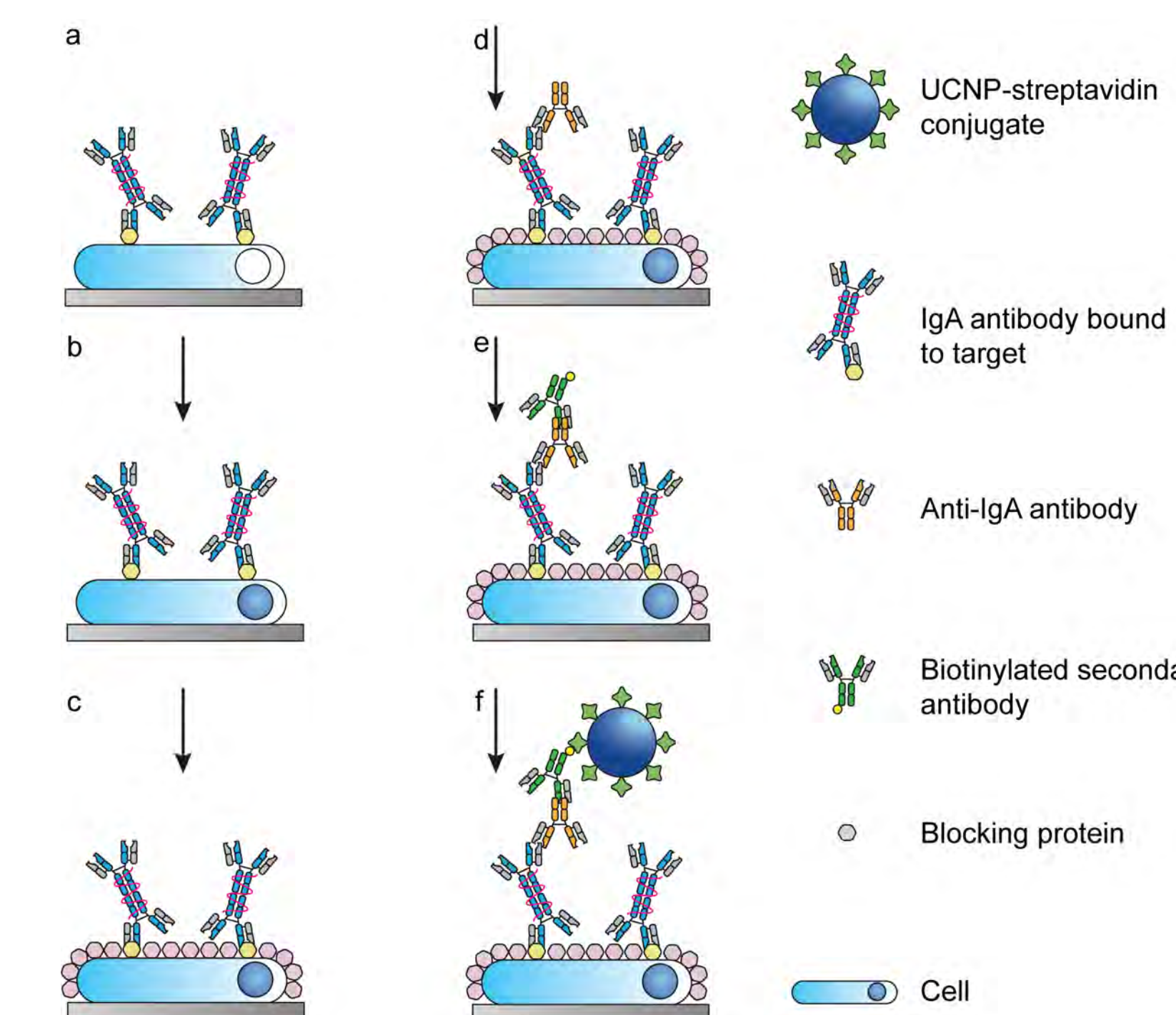
Results:

Analyses of the images showed that the UCNP-based technique provided diagnostically interpretable images of IgA labelling, comparable with the images from the sections stained with conventional IP techniques.



The same glomerulus photographed:
A) Brightfield image
B) Inverted brightfield image
C) Brightfield and UCNP image overlay
D) IgA IHC

How does UCNP work?



- Sample with IgAs bound to their target
- Antigen retrieval & haematoxylin counterstaining
- Blocking of non-specific binding sites and endogenous biotin
- Incubation with a primary rabbit anti-IgA antibody
- Binding of the biotinylated anti-rabbit antibody
- Attachment of SCIZYS Erbium-SA UCNPs

Advantages	Disadvantages
Near-infrared excitation (980 nm) reduces light scattering and assures high tissue penetration	UCNPs are large in size compared to antibodies
Anti-Stokes emission prevents autofluorescence	Scanning time longer because an additional image is taken
No photobleaching over long time with continuous excitation ¹	Chromogenic counterstain is mandatory for focusing
Brightfield and luminescence image are made consecutively to assure perfect overlay of the brightfield and luminescence images	Currently, protocol 30 min longer compared to HRP/DAB
Performed on FFPE sections. No requirement of fresh/frozen tissue Produces digital images	Currently, biotinylated primary or secondary antibody necessary Currently, scanner only available with 20x magnification
Can change colour/contrast to suit pathologists	Currently, scanner can hold four slides
No enzymatic amplification step necessary	

1. [https://doi.org/10.1039/C9NR10568A\(SI\)](https://doi.org/10.1039/C9NR10568A(SI))

Conclusions:

UCNP-based IHC has the potential to become established in renal diagnostic practice and further multicentre studies using the full range of diagnostic antibodies is needed. Comparison with IF, if favourable would also negate the need for an additional core of fresh renal tissue.