Lateral Flow Development Services

FROM CONCEPT TO COMMERCIAL SUCCESS:
TOGETHER WE CAN CREATE A BRIGHTER FUTURE

stream bio
DIAGNOSTICS

Contents

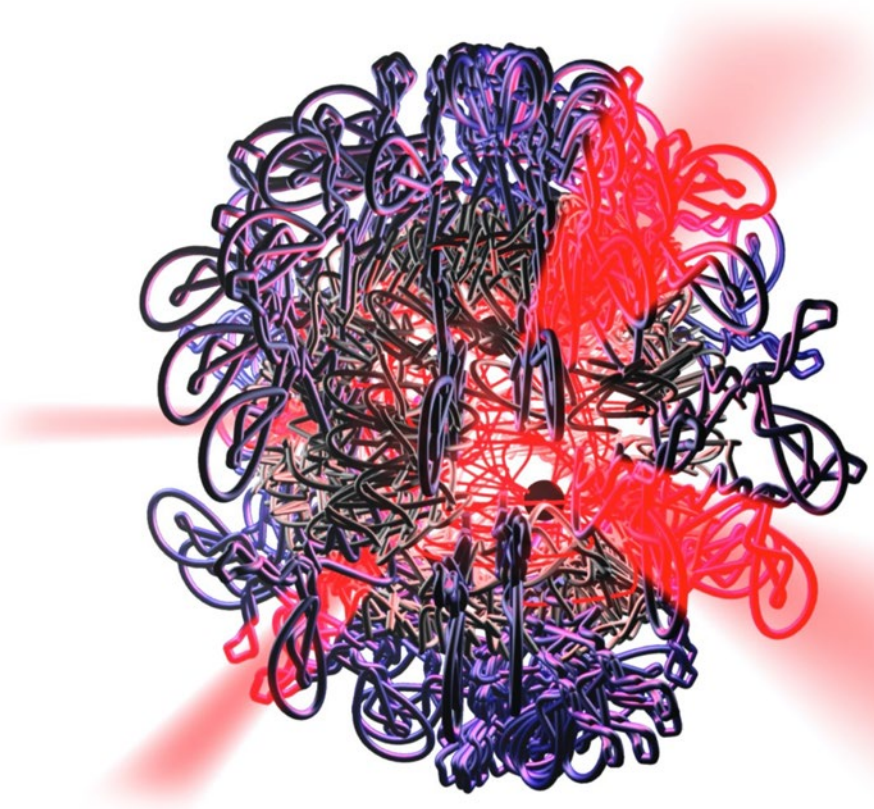
We offer a phased development service from proof of concept through to validation, small-scale manufacture and technical transfer to a CMO for large-volume production. Our service utilises our novel ultra-sensitive technology – Conjugated Polymer Nanoparticles (CPNs™), which have superior properties to colloidal gold and europium chelates. See page 5.

CPN™ Diagnostic Development Service

▶ #DiscoverBright
▶ #DiscoverContrast

See how we can help you outshine the competition

At Stream Diagnostics, a division of Stream Bio, we tailor our novel nanotechnology to help you deliver a lateral flow test (LFT) that meets your unique needs and outshines offerings available from other competitors, with the following features:



- Extraordinary detection capability
- Clear, precise results to accurately detect and quantify antigens
- Test signals that can be read visually, or analysed using our own, or commercially available readers
- Original equipment manufacturer options available
- Multiple colours for multiple targets and the ability to multiplex on one test strip

Lateral flow tests: an overview

An LFT is an immuno-sandwich assay that can detect the presence of a target antigen in a liquid sample (e.g., saliva, mucous, blood) in as little as 5-10 minutes, playing a vital role in the rapid diagnosis of infections and diseases.

Over the years, LFTs have been commonly used in human and veterinary medicine, as well as food and environmental safety, and can be read visually or with a reader. Outside of clinical and industrial settings, the earliest examples of home lateral flow tests were common pregnancy tests.

Throughout the ongoing COVID-19 pandemic, home LFTs have become a popular tool to help identify carriers and manage the spread of SARS-CoV-2.

Moving forward, we anticipate that LFTs will continue to help address public health challenges and help stratify patients at the point of care.

Figure 1 - CPNs can be detected and read on a test line using a variety of readers, including Stream's reader: Claritas

Our CPN LFTs have the potential to be used as critical rapid diagnostic tests that will aid in the early diagnosis and treatment of a variety of infectious and non-infectious diseases. Additionally, our own fluorescent lateral flow reader, Claritas, with CPN LFTs, can offer PCR-level sensitivity detection in minutes in any environment.

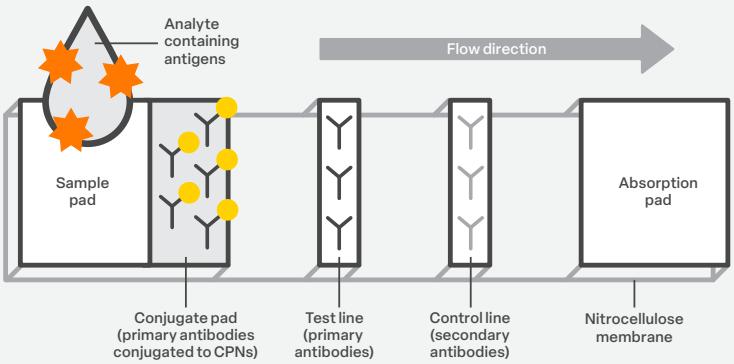


Figure 2 - The basic structure of an LFT. As the analyte travels through the strip via capillary flow, antigens in the analyte will attach to antibodies conjugated to CPNs, the antibodies in the test line and the control line. The presence of visible test and control lines helps to confirm a positive test result.

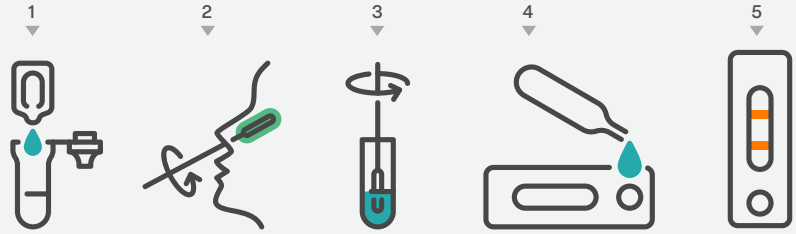


Figure 3 – Typical steps involved when conducting a rapid LFT at home: **1)** Transfer of extraction buffer into a tube, **2)** Sample collection using a swab, **3)** Dissolving of the specimen on the swab in the extraction buffer in the tube, **4)** Addition of drops from the tube in the designated well of the test cassette. **5)** Test results are determined based on the lines present on the strip.

Conjugated Polymer Nanoparticles vs gold nanoparticles & europium chelates

Our CPNs absorb 40% more light than conventional gold nanoparticles. In LFTs, this facilitates improved contrast and visibility of the test line, regardless of illumination. Therefore, CPN-based LFTs have a higher detection sensitivity than gold.

Our CPNs produce more intense signals that can remain stable over months, and even years. Their outstanding light absorbance, fluorescence and robustness make them ideal for lateral flow assays.

CPNs exhibit luminescence or fluorescence with emission wavelengths covering the visible and infrared (IR) spectrum.

Fluorescent LFTs are intrinsically better than visual assays. Using a fluorescent signal rather than a visual signal significantly improves the detection capability of LFTs. For example europium (III) chelate has a "300-fold better sensitivity compared to colloidal gold."[1]

CPNs fluoresce around 100 times brighter than europium (III) chelates, further enhancing performance. Compared to europium chelates in LFTs for Influenza A/B, CPNs can improve test signal and detection limit (Figure 6).

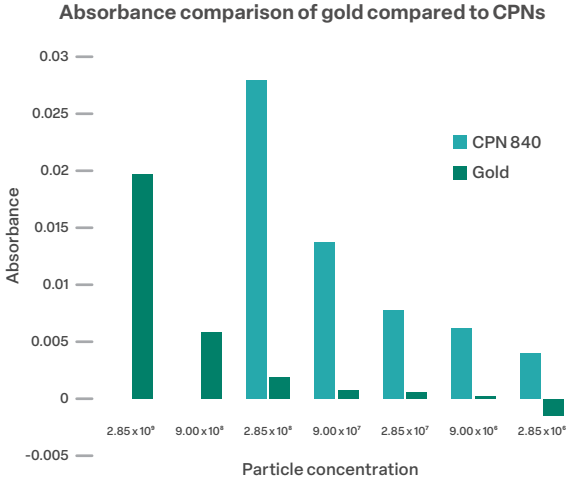


Figure 4 - Serial dilution of gold compared to CPNs at matching particle concentrations. CPN 840 has significantly more intense absorbance per particle.

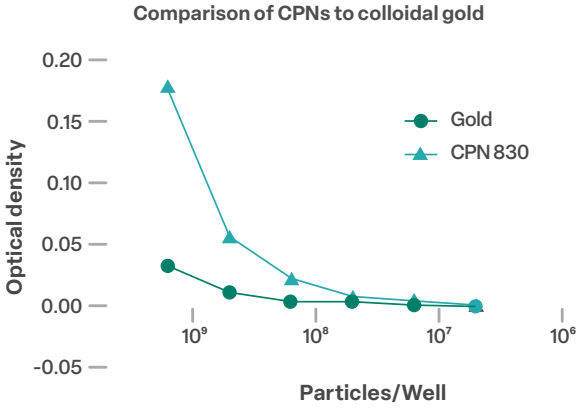


Figure 5 – Optical density of decreasing quantities of CPN 830 and 40 nm colloidal gold

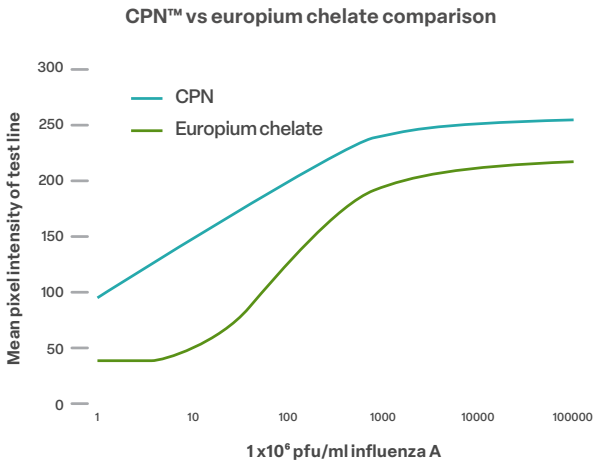


Figure 6 - Lateral flow strips produce stronger signals than europium chelate in a LFT for influenza A

1 Juntunen E, Myyryläinen T, Salminen T, Soukka T, Pettersson K. Performance of fluorescent europium(III) nanoparticles and colloidal gold reporters in lateral flow bioaffinity assay Anal Biochem. 2012; 428(1):31-8 doi: 10.1016/j.ab.2012.06.005

Visually read lateral flow tests

Case study: Ovalbumin

With our CPNs, you can achieve multi-coloured, multiplexed test strips that generate clear, precise results to detect multiple antigens. Thanks to the CPNs' extremely high light absorbance, which is 40% higher than colloidal gold, and signal intensity, test results can be read visually.

In this case study, visual CPNs LFTs achieved a sensitivity of 0.1 ng/ml.

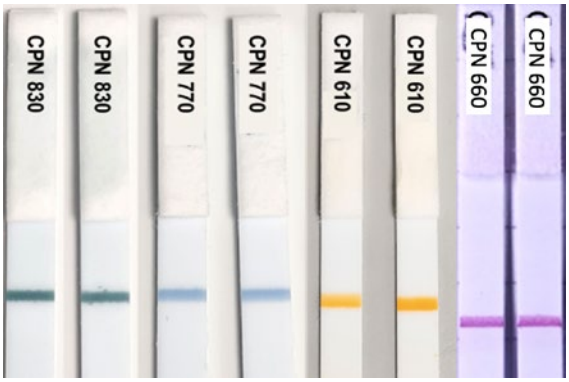


Figure 7 - Multi-coloured LFTs using CPN 830, CPN 770, CPN 610 & CPN 660 to detect ovalbumin

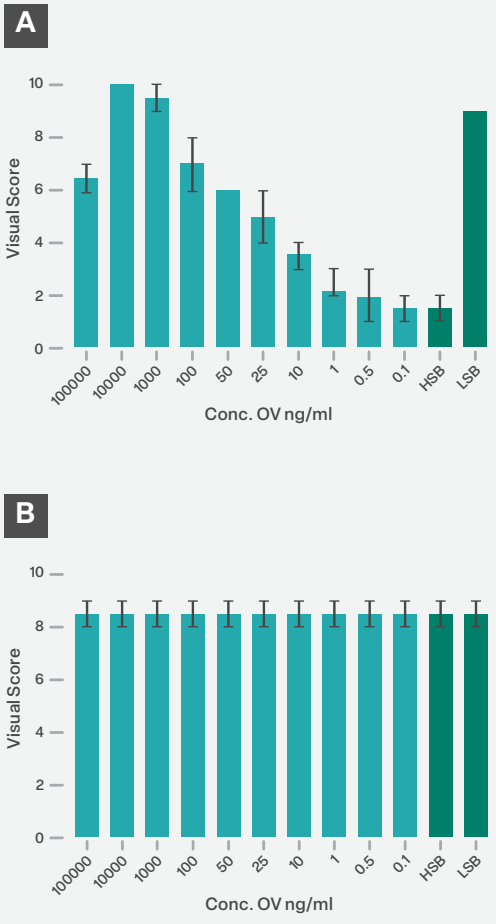


Figure 8 – Test line (panel A) and control line (panel B) intensity following challenge with ovalbumin at concentrations ranging from 0.1 ng/mL to 100 µg/mL (n=6).

Dark green columns show buffer controls (n=3). Columns depict the mean visual score ± the

By utilising multiple CPN colours, we can develop LFTs to detect multiple antigens on one test strip or even with a traffic light system that can indicate the severity of an infection.

When analysed using our Claritas reader or other commercially available readers, the target can be accurately quantified.

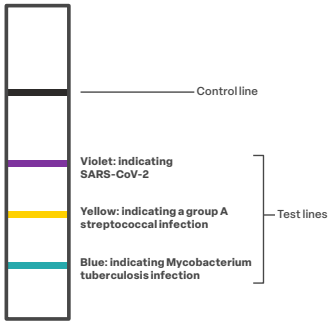


Figure 9 – A diagram of a multiplexed, multi-coloured test strip that could help clinicians diagnose patients. Based on the colour of the test line that appears, they can offer tailored treatment.

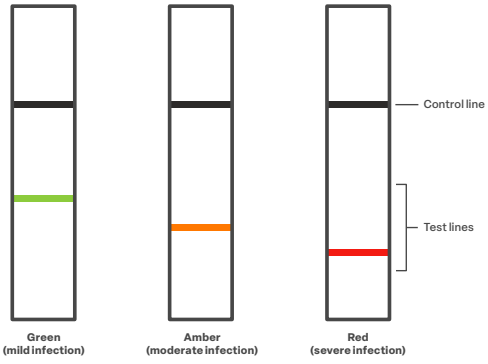


Figure 10 - A diagram of how an LFT with a traffic light system can indicate the severity of an infection

Fluorescent lateral flow tests

Case study: SARS-CoV-2 lateral flow assays

Our CPNs are an ideal detection agent for infectious diseases such as SARS-CoV-2. As part of the COVID-19 pandemic response, we applied our technology to the detection of SARS-CoV-2 infections and demonstrated considerable success.

Conventional LFTs were shown to have a detection capability of $TCID_{50}$ equal to 1000 or more. When utilised with our proprietary Claritas reader, our CPN-based SARS-CoV-2 LFT demonstrated a PCR equivalence of approximately 30-35 cycles or a $TCID_{50}$ of less than 100. In the early stages of the pandemic, our test could have potentially detected asymptomatic and pre-symptomatic cases 3-4 days before symptoms present.

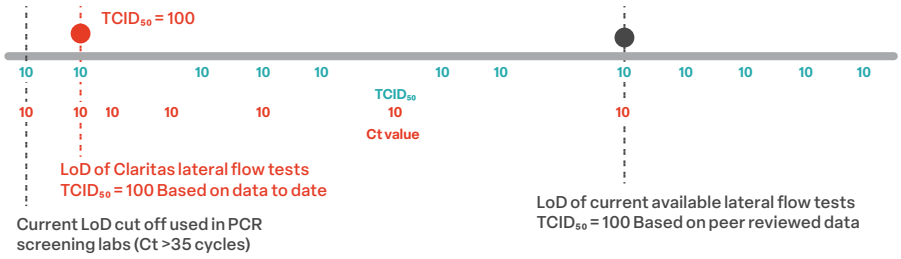


Figure 11 - Limit of detection of CPN-based SARS-CoV-2 lateral flow tests (red) compared to the limit of detection of current LFTs[3]

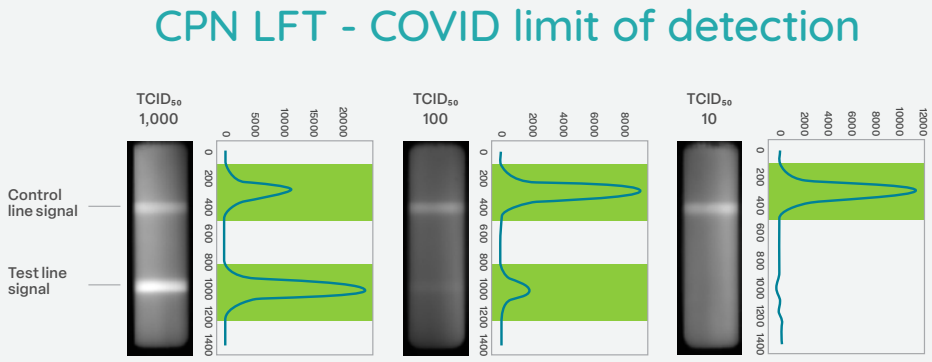


Figure 12 - Limit of detection of CPN-based SARS-CoV-2 lateral flow tests[4]. Clear signal seen to $TCID^{50}$ = 100 by the Claritas LFT reader

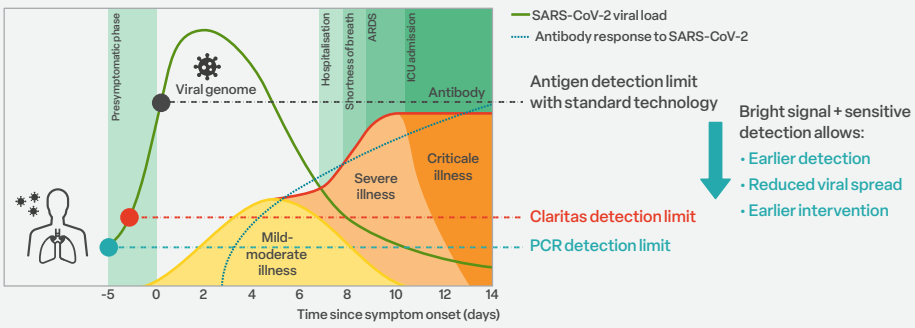


Figure 13 – How CPN LFTs and Claritas could help provide PCR-equivalent test results[4]

3 *Arnaout R, Lee R, Lee G, Callahan C, Yen C, Smith K, Arora R, Kirby J. SARS-CoV2 Testing: The Limit of Detection Matters bioRxiv 2020; doi: https://doi.org/10.1101/2020.06.02.131144

4 Figure adapted from: Cevik M, Kuppalli K, Kindrachuk J, Peiris M. Virology, transmission, and pathogenesis of SARS-CoV-2 BMJ 2020; 371 :m3862 doi:10.1136/bmj.m3862

Fluorescent lateral flow tests

Case study: high-sensitivity C-reactive protein lateral flow assays

C-reactive protein (CRP) can act as a biomarker for inflammation in the body, high levels of which indicate disease or infection.

Using our CPNs, we developed CRP tests that are up to 800 times more sensitive than FDA-approved CRP tests currently available on the market[2].

By applying our novel technology to the detection of lower levels of specific analytes, biomarkers and diseases of interest, these CPN LFTs could bring lab-based sensitivity to the point of care.

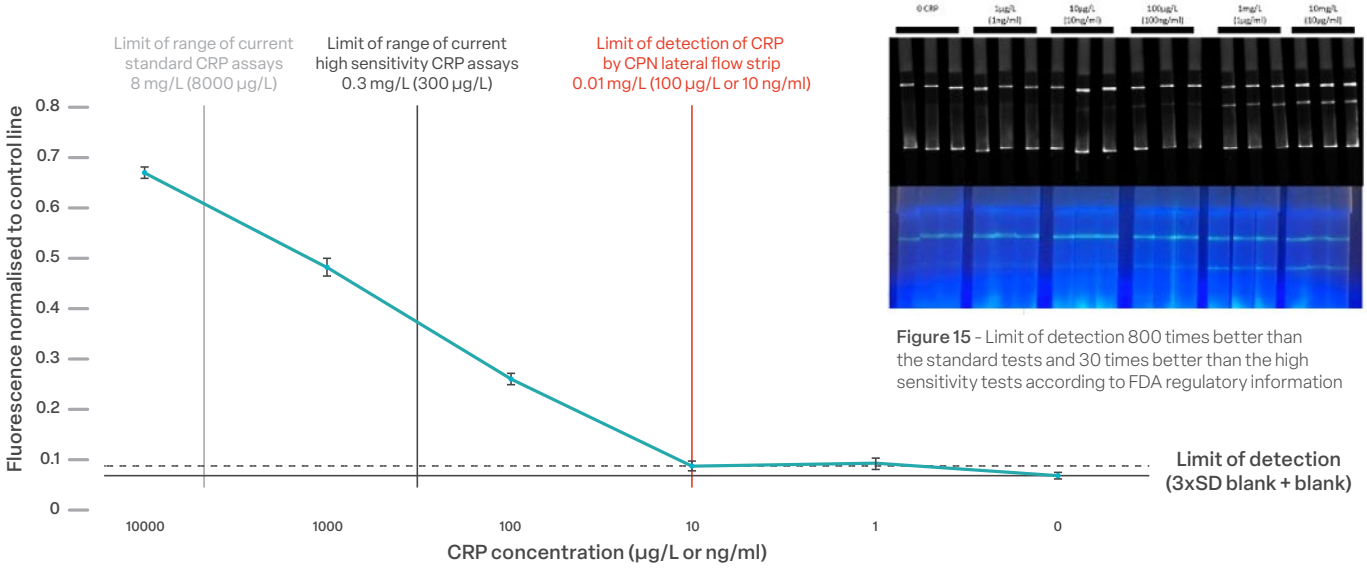


Figure 15 - Limit of detection 800 times better than the standard tests and 30 times better than the high sensitivity tests according to FDA regulatory information

Figure 14 - Comparison of limits of CRP detection of FDA-approved assays and CPN assays.

Using fluorescent probes such as CPNs in LFTs enables powerful signal amplification. They can help to detect even the lowest levels of target analytes. CPN LFTs could therefore help with the earlier diagnosis and identification of asymptomatic carriers of infectious diseases (see page 7), which is key to controlling the spread of pathogens and administering immediate treatments.

2 US Drug and Food Administration. Review Criteria for Assessment of C-Reactive Protein (CRP), High Sensitivity C-Reactive Protein (hsCRP) and Cardiac C-Reactive Assays 2018; FDA-2020-D-0957

Optional LFT reader & OEM options

At Stream, we will work with you to tailor a solution to your needs. If you have plans to use your own, or a third party's instrumentation, we customise our CPNs accordingly.

Alternatively, we can offer our reader, Claritas, specifically developed to analyse CPN lateral flow tests in a point-of-care environment, from the lab to the field. A fluorometric camera (sensitive up to 1000nm, across the visible and near infra-red spectrum) captures an image of the test line and measures the intensity of the signal. Algorithmic analysis then determines the level of quantification and displays it on a full-colour touch screen. A batch mode analysis allows a test to be read and processed every 5 seconds, even in the most challenging settings.

Early-stage fluorescent reader development demonstrated a detection capability that could resolve as few as 600 individual nanoparticles (see figure 17). This indicates a potential assay detection capability of 600 individually bound antigen protein targets. It should be noted, however, that factors including targeting molecule (e.g. antibody/oligo) binding events, assay flow rates, and background fluorescence (noise) influence an assay's limit of detection.

- Weight 1.5 kg
- 6 hr rechargeable battery
- Waterproof, drop-proof (1 m) casing
- Digitally enabled - data storage and QR codes
- Digital connectivity - WiFi/Bluetooth/GPS/3G or 5G options

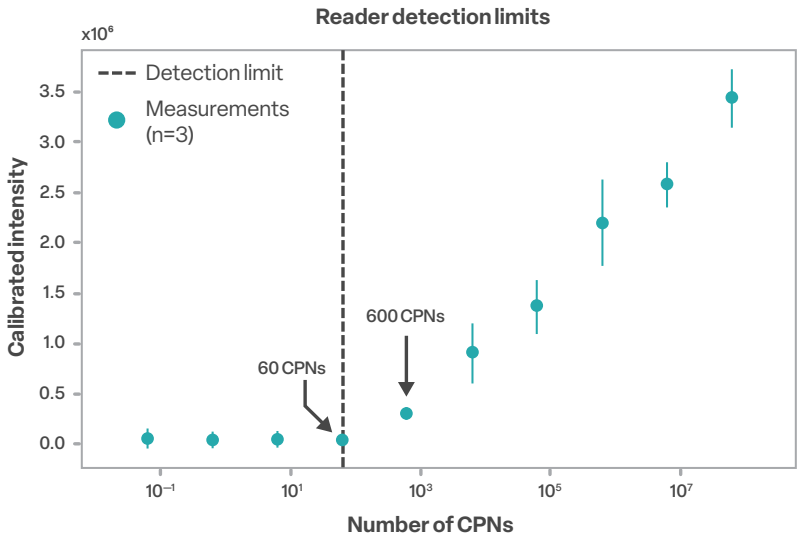
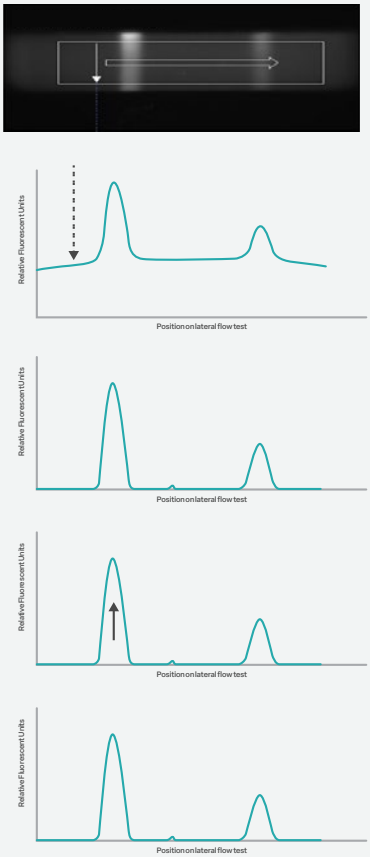


Figure 16 – Fully quantitative results using from 600 CPNs, potentially identifying 600 protein targets (NB: This is a theoretical detection limit, assay development, target binding events, and other factors influence final sensitivity of an assay)

Figure 17 – Schematic illustration of the steps performed for the image analysis



Eliminating background noise with infrared lateral flow assays

CPNs that fluoresce in the near-infrared (NIR) window emit light that penetrates the surrounding materials and fluids, such as blood or lateral flow membranes, effectively creating an environment with minimal background noise.

This is the next boundary for lateral flow, and one currently being pushed and developed by Stream Diagnostics.

When using IR CPNs in LFTs, background interference or noise can be significantly reduced, which can take a test's detection sensitivity to a new level. IR CPNs can deliver LFTs with exceptional detection sensitivity.

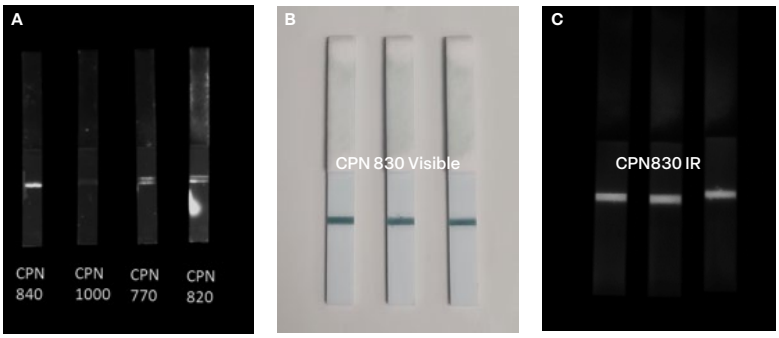


Figure 18 – A: Lateral flow tests labelled with IR CPNs+anti-mouse IgG, imaged with IR camera, ImageQuant 800 detector (ex/em = 785/830 nm). Clear signals at the test/control lines & little background signal detected from the nitrocellulose strip. B/C: CPN 830: Control line is an anti-mouse that binds the mouse primary antibody on the surface of the CPN. B: ImageQuant used visible light for absorbance image. C: IR Image, ImageQuant used ex/em = 660/836 BP46 nm

More about our reagents

Our CPNs are powerfully bright fluorophores with many advantages over conventional dyes and probes.

- Provide immense brightness and contrast
- Extraordinary photo-, thermo- and chemical stability
- Available in a wide range of wavelengths on the visible and IR spectrum

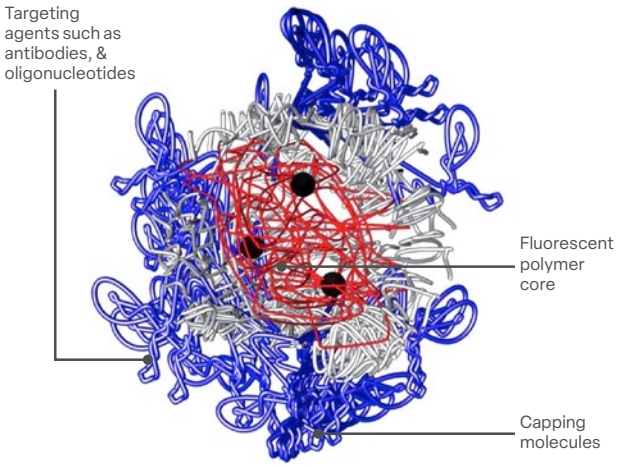


Figure 19 - Structure of a CPN

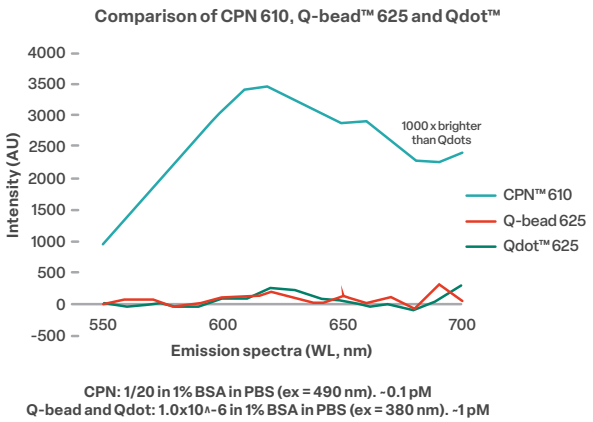


Figure 20 – Experimental data from The Ozcan Research Group, University of California, Los Angeles, which shows that CPN™ 610 (Orange) is up to 100-1000x brighter than equivalent Q-beads and Qdots. Data measured on a standard ELISA plate reader with excitation and emission optimised for each fluorophore."

CPNs have an intense fluorescence that reaches into the IR (420-1000 nm). Different surface chemistries are available, such as carboxyl, maleimide, streptavidin and alkyne, with rapid (30/60 minutes) antibody & oligonucleotide conjugation kits.

In addition to lateral flow, CPNs can be utilised in ELISA, western blot, cell labelling and microscopy.

They are 1000 times brighter than quantum dots and 100 times more sensitive than europium (III) chelates in a Flu A/B case study (see page 5). The key to the robustness, brightness and, in turn, sensitivity is their double-back-boned conjugated polymer core structure, which has an exceptionally high extinction coefficient (absorption of photons). This is in addition to their average quantum yields.

Brightness = Quantum Yield x Extinction Coefficient

CPN	ex/em	CPN	ex/em
CPN 420 (Violet)	390/420	CPN 610 (Orange)	480/610
CPN 435 (Indigo)	390/435	CPN 660 (Red)	540/660
CPN 475 (Blue)	390/475	CPN 680 (Red)	400/680
CPN 510A (Green)	455/510	CPN 770 (IR-I)	610/770
CPN 510B (Green)	400/510	CPN 820 (IR-I)	640/820
CPN 530 (Green)	455/530	CPN 830 (IR-I)	610/830
CPN 550 (Yellow)	470/550	CPN 840 (IR-I)	630/840
CPN 580 (Orange)	488/580	CPN 1000 (IR-II)	750/1000

Figure 21 – CPN product range and wavelengths

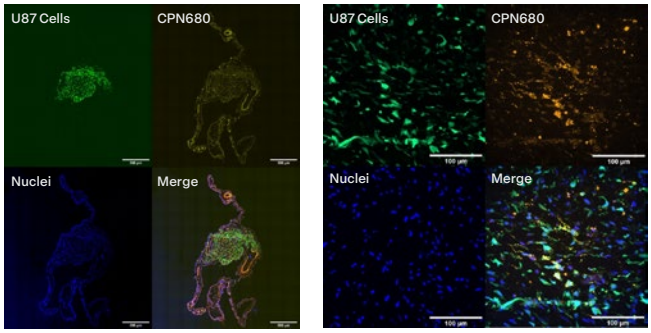


Figure 22 - Glioblastoma cancer cells were labelled with CPN 680 (orange) for 24 hours in vitro and then implanted into the chick embryo chorioallantoic membrane at embryonic day 7. Tumours were dissected from the embryo at embryonic day 14, frozen and sectioned. (A) Images show glioblastoma cells (green) remain labelled with CPN 680s (orange) in a highly proliferating environment from single cells to a tumour mass. (B) Images were taken at 40 times magnification to confirm CPNs remained within tumour cells. Images taken in collaboration with the University of Liverpool.

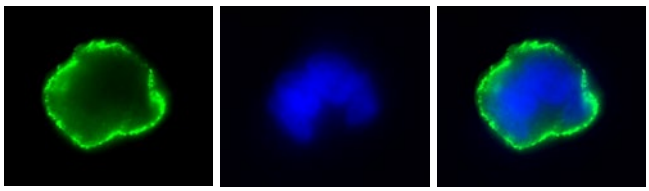
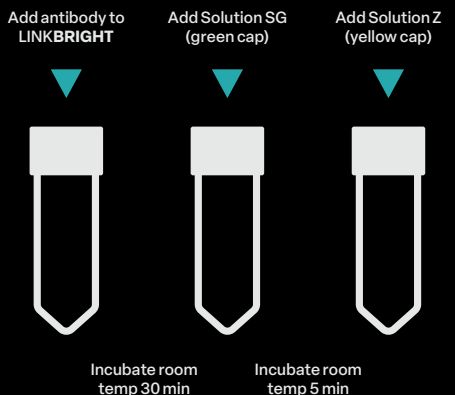


Figure 23 - CPN 510B linked to anti-carcinoembryonic antigen (CEA) affimer targeting colorectal cancer cells, imaged by the University of Leeds

LINKBRIGHT™ Amine IgG Antibody Conjugation



Our phased lateral flow test development process

Phase 1: Proof of principle (4-week turnaround)

- Sourcing and testing of antibodies to assess their suitability for use in LFTs

Phase 2: Determination of assay feasibility (4-week turnaround)

- Building on the work to date and continuing to work on assay development

Phase 3: Optimisation of assay (8-week turnaround)

- Improving assay dynamics and sensitivity
- Integrating the test strip into a cassette to produce the final product concept for review

Phase 4: Assay validation (4-week turnaround, 6-24 months for stability tests)

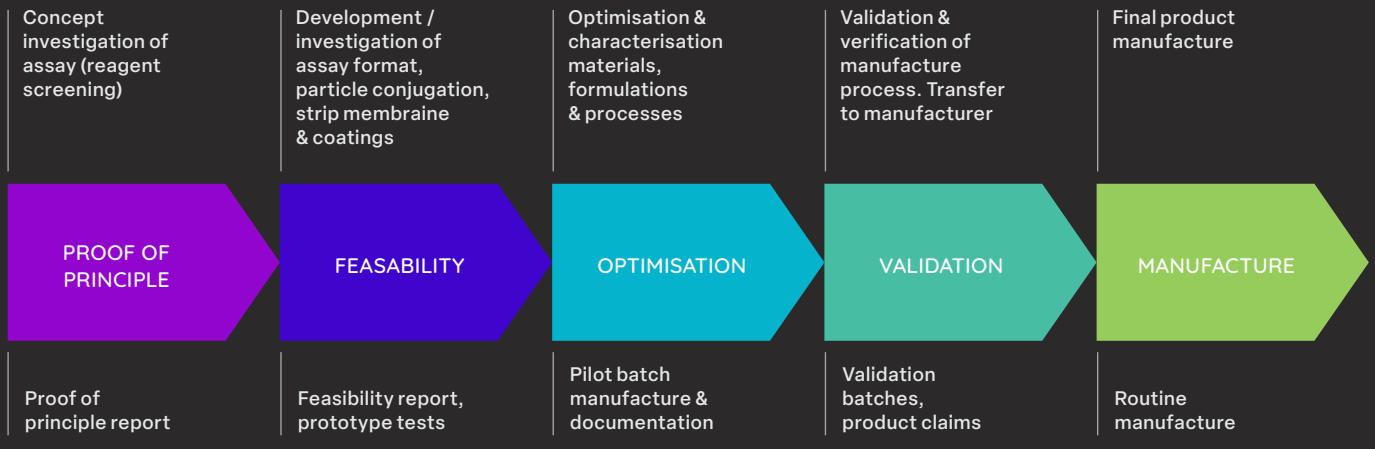
- Validating devices to ICH standards
- Ensuring tests will demonstrate a shelf life and extension plan of over 2 years

Phase 5: Manufacture of test strips (2-week turnaround)

- Providing training and a full technical report for the assays
- Transferring production methodologies to contract manufacturers
- Performing small-scale manufacturing (10,000 devices a month), if required

Regulatory Support:

In addition to our development services, we offer a range of quality and regulatory consultancy through our partners. Our end-to-end service offering covers UK/EU/US: Preparation of a regulatory strategy plan, preparation and submission of technical documentation, preparation and submission of FDA 510(k) pre-market notification.



For more information, visit www.streambio.co.uk

Contact us today

General enquiries
info@streambio.co.uk

Technical enquiries
dermott.ocallaghan@stream.co.uk

Sales enquiries
sales@streambio.co.uk
andy.chaloner@streambio.co.uk
steve.self@streambio.co.uk

@Stream_Bio
www.linkedin.com/company/streambiosciences

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