IMAC increases sensitivity in malaria diagnostics

Immobilized metal affinity chromatography (IMAC) is a popular method for protein purification, particularly for recombinant proteins fused to a polyhistidine tag. But IMAC can be used in many other applications. Its high binding capacity towards the malaria biomarker protein HRP2 makes it an excellent enrichment method to increase sensitivity in malaria point of care diagnostic assays.

The challenge of low-level malaria infections

According to the World Health Organization (WHO), about half the world's population—3.2 billion people—are at risk for malaria [1]. While low-level infection is often asymptomatic, it prevents the elimination of the disease because infected individuals act as parasite reservoirs. Rapid diagnostic tests based on lateral flow assays (LFAs) have been recently introduced as point-of-care (POC) tools, as they are easy to use and relatively inexpensive. However, LFAs do not provide the sensitivity needed to detect low-level infections [1].
Meanwhile, signal-enhancing methods with higher sensitivity have been developed, but their implementation is not always straightforward. Governmental approval, training of healthcare workers, commercialization, and deployment are issues that need to be overcome. In addition, methods suffer from long assay times, the need for cold chains, or incompatibility with whole blood samples.

The idea: Combine two proven methods...

David Wright and his team at Vanderbilt University in Nashville, TN, have established a different strategy for the detection of low parasite levels and are collaborating with the Macha Research Trust in Zambia for clinical deployment. Instead of developing a novel method from scratch, they combined two proven technologies: enrichment of the main biomarker protein, HRP2, and established LFA tests [2]. In their approach, they exploited the affinity of the histidine-rich HRP2 malaria biomarker protein to immobilized metal affinity chromatography (IMAC). Magnetic-based IMAC chemistry was shown to enrich HRP2 biomarkers from citrate phosphate dextrose (CPD)-stabilized lysed whole blood in a simple manual device that can be integrated with the LFA assay. The procedure is robust, does not require cooling, and uses gentle elution conditions which are assay-compatible.

... then optimize performance...

To further optimize sensitivity, cost, and processing time, the authors tested magnetic IMAC beads from different vendors. Using Cube Biotech Ni-NTA MagBeads, enrichment conditions were raised to 80-85% capture of HRP2 from samples on a wide range spiked with 65-4000 parasites per µL blood (Figure 1A).
As reports suggested a higher affinity of divalent zinc (Zn^{2+}) than that of the typically used nickel, copper or cobalt, the relationship between divalent metal species, recovery rates and processing time was investigated in a next step.

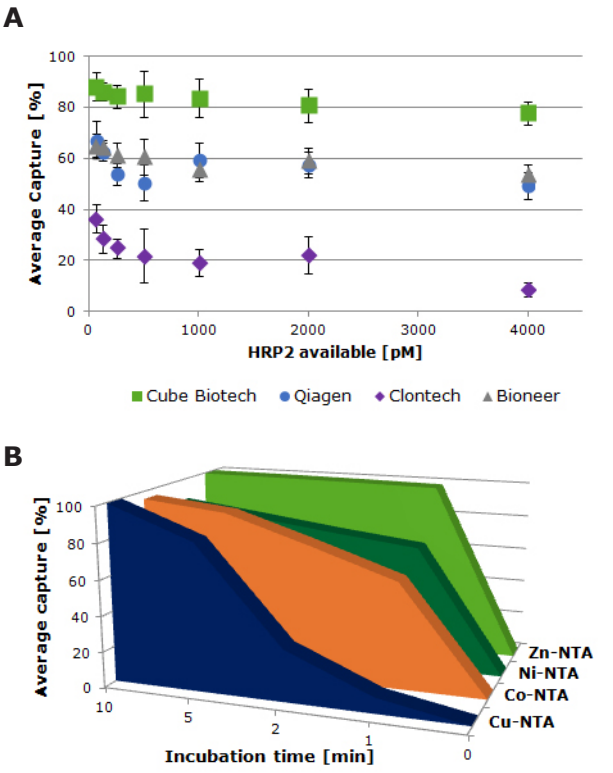

Figure 1: Cube Biotech Zn-NTA MagBeads are optimally suited for HRP2 capture.
A: Cube Biotech Ni-NTA MagBeads, QIAGEN Ni-NTA Magnetic Agarose Beads, Bioneer AccuNanoBead Ni-NTA, and CloneTech His60 Ni Magnetic Beads were evaluated for bead performance.
Standardized bead volumes were added to lysed whole blood spiked with *P. falciparum D6* parasite culture preparations. After 10 min incubation, magnetic beads were separated, supernatants were collected, and the residual HRP2 not captured by the beads was quantified using an enzyme-linked immunosorbent assay (ELISA). Using Cube Biotech Ni-NTA MagBeads, enrichment conditions could be significantly improved from 80% HRP capture in blood samples spiked with 200 parasites /µL to 80-85% capture of samples on a wide range spiked with 65-4000 parasites per µL blood. This effect was achieved with only 2.1 µL of magnetic bead solution instead of the 20 µl that were previously needed.

B: Cube Biotech beads equipped with four different divalent metals were used to determine HRP2 capture as a function of processing time. Experimental conditions were the same as in A.

...and let zinc-NTA do the trick

Indeed, magnetic beads with Zn(II)NTA surface functionalization were found to capture 99.7 ± 2.7% of the available HRP2 at 10 min mixing time, more than any other divalent metal. At mixing times of only 1 minute, as advised for POC diagnostic systems, the surface functionalization was found to have an even greater impact: Zn(II)NTA functionalization enabled the capture of 98.2 ± 2.7% of the available HRP2, providing by far the best performance (Figure 1B). Thus, employing Cube Biotech's improved magnetic bead solid phase together with optimal Zn(II)NTA surface functionalization allowed for a 10-fold reduction in mixing time, while also increasing HRP2 capture by 20% compared to previous methods. Last but not least, this performance increase was achieved at an additional magnetic bead cost of only 0.09$ per assay, which is the lowest among the materials tested (Figure 2).

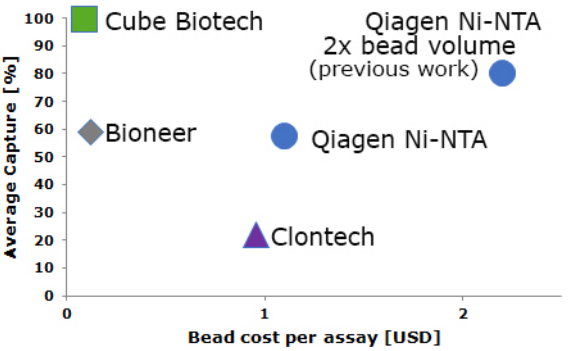

Figure 2: Cube Biotech provides most efficient HRP2 capture at lowest material cost.
5.5 mm^3 magnetically packed volumes of IMAC beads manufactured from four different manufacturers were evaluated as described in Figure 1.
A one minute mixing cycle was used for Cube Biotech Zn(II)NTA whereas ten minute mixing cycles were used for the other three IMAC magnetic beads.

Agarose as alternative matrix

While the magnetic bead-based approach is highly specific and sensitive, it requires some equipment which is not always available at the point of care. Therefore the authors also investigated an enrichment method based on simple manual pipette tips filled with non-magnetic IMAC agarose [(3)]. Again, Cube Biotech Zn-NTA Agarose demonstrated optimal binding and elution properties and the highest recovery of HRP2 protein from blood samples compared to other materials. The observed increase of signal intensity and sensitivity was almost as significant as for the magnetic bead-based approach. Both simple, inexpensive enrichment procedures hold significant potential for a fast implementation in malaria diagnostics, paving the way to a complete eradication of the disease.

The optimized setup fulfills the ASSURED requirements postulated by the WHO:

Affordable:	minimal additional cost per assay
Sensitive:	increased detection from 55% to 95% of infectious reservoir <200 parasites/µL, compatible with whole blood samples, minimal sample carryover volume, removes interferents that can induce false positive results (e.g., autoantibodies or anti-mouse antibodies)
Specific:	compatible with 5 commercially available assays
User-friendly:	using existing system with convenient mag bead separation
Rapid and **R**obust:	only 3 min additional processing time in a single-use sample tube
Equipment-free:	3D printed device, battery-powered mixer
Deliverable:	Easy to deploy, no cooling needed, >1 year shelf life, beads can be lyophilized

Featured products:
PureCube Zn-NTA MagBeads (Cat. No. 32405-Zn)
PureCube Zn-NTA Agarose (Cat. No. 31403-Zn)

Literature Cited:
1. World Health Organization, Malaria Rapid Diagnostic Test Performance: Results of WHO Product Testing of Malaria RDTs: Round 6 (2014-2015) WHO, 2015)
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