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ENHANCED PERFORMANCE OF A LATERAL FLOW ASSAY

Use of a novel conjugate blocking technology to improve performance of a gold nanoparticle-based lateral flow assay

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ABSTRACT

BBI has developed a novel conjugate blocking technology - *Morffi* ™ - with the potential to enhance the sensitivity of lateral flow immunoassays. In this paper, we demonstrate the increased sensitivity that can be achieved using this new blocking technology, comparing it with traditional BSA blocking in a brain natriuretic peptide assay. The results show that this proprietary technology offers a 10-fold improvement in the limit of detection over the BSA-blocked control, highlighting its potential for enhancing both current and future lateral flow tests.

INTRODUCTION

Lateral flow immunoassays (LFIs) are relatively simple yet powerful diagnostic tools. Used for an extensive range of laboratory and point-of-care (POC) applications, the simplicity of these tests allows them to be performed by skilled laboratory staff and laymen alike.[1] LFIs offer accessible, low-cost diagnostics, fulfilling many of the World Health Organisation's ASSURED (Affordable, Sensitive, Specific, User-friendly, Reliable and robust, Equipment-free, Deliverable to end-users) characteristics.[2]

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LFIs rely on the specific interaction between the analyte of interest and an immobilised binding partner (e.g. antibody, antigen or enzyme). The binding partner is conjugated to a reporter label which is used to indicate the presence or absence of the target molecule, often represented by a coloured line in visual LFIs. During manufacturing, conjugation of the binding partner to the reporter label is unlikely to achieve complete saturation of the label surface, even when the binding partner is in molar excess. Non-specific binding of molecules from the sample matrix to these unoccupied conjugation sites during the assay reduces its sensitivity and/or specificity. These unoccupied sites may also allow cross-linking between reporter molecules, leading to aggregation and further reducing assay sensitivity.[3]

The performance of LFIs can be improved through the use of a 'blocking' agent to prevent non-specific binding. These agents can be used to decrease or completely eliminate the availability of free binding sites on the label, without taking part in any assay-specific reactions. This process is widely known as 'conjugate blocking', and is commonly performed following immobilisation of the analyte binding partner. Blocking agents can be bound either passively or covalently to the surface of the reporter label, and over 95 per cent of reporter conjugates currently produced for use in LFIs are blocked with bovine serum albumin (BSA).

Using BSA in this way can reduce non-specific binding of molecules to the reporter label, as well as cross-linking between reporters, increasing sensitivity and specificity. However, depending on the application, the use of BSA presents a number of problems, such as cross-reaction with human serum albumin-binding antibodies and bovine- and other animal-origin targets, as well as lot-to-lot variability. The relatively large size of the BSA molecule (67 kDa) can also lead to steric hindrance for the detection or immobilisation of small molecules, by reducing the availability of the binding partner to the target molecule. Several alternative blocking agents are available – such as dry milk proteins, polyethylene glycol and fish gelatin – but these still suffer from lot-to-lot variability and non-specific binding issues.

This proprietary blocking process does not require any further adjustments to the conjugate manufacturing parameters.

BBI has developed a novel technology - *Morffi* - which overcomes these issues. This proprietary blocking process does not require any further adjustments to the conjugate manufacturing parameters and, due to its size in comparison to BSA, can improve the availability of analyte-specific binding partners on the surface of the label, increasing the sensitivity or signal intensity of the assay. The potential of this technology is demonstrated by an LFI using a 40 nm gold nanoparticle reporter to detect brain natriuretic peptide (BNP).

MATERIALS AND METHODS

BBI uses an in-house developed 'BNP-32' LFI as a model system for the assessment of new technologies. The assay combines the company's proprietary colloidal gold label with HyTest detection and capture antibodies and commercially available nitrocellulose membrane (card-laminated) and pad materials.

For this experiment, 40 nm BBI colloidal gold was passively conjugated to an anti-BNP detection antibody under predetermined optimal conditions. Following antibody immobilisation, unconjugated sites on the gold particles were blocked with an excess of either a BSA control or BBI's patent-pending *Morffi* blocking technology. The reporters were then normalised to the same optical density (OD1 at 520 nm) in phosphate buffered saline (PBS) + 1 % TWEEN® 20 before use.

The capture anti-BNP antibody was immobilised onto the nitrocellulose membrane, dried and laminated onto wick pads. The assembled membrane/wick pad cards were then cut into 4 mm wide test dipsticks.

The assay strips were tested against a range of antigen concentrations prepared in PBS at pH 7.2. A 20 µl aliquot of each antigen concentration was premixed with 20 µl of either BSA- or *Morffi*-blocked reporter particles prior to addition of the dipsticks. Following a 15 minute incubation at room temperature, test dipsticks were read using a CAMAG TLC Scanner 3 at 520 nm. Five replicates were performed at each concentration.

The limit of detection (LoD) for the BNP-32 assay was then determined for each blocking technology, based on double the standard error (SE) above and below the mean signal for each concentration.

RESULTS

Improved sensitivity

Use of the novel blocking technology results in a dramatic improvement in the LoD compared to the BSA-blocked control (Figure 1). The LoD achieved using *Morffi* was 0.08 ng/ml, compared to 0.8 ng/ml for the BSA-blocked control, representing a 10-fold increase in the LoD for the BNP-32 assay.

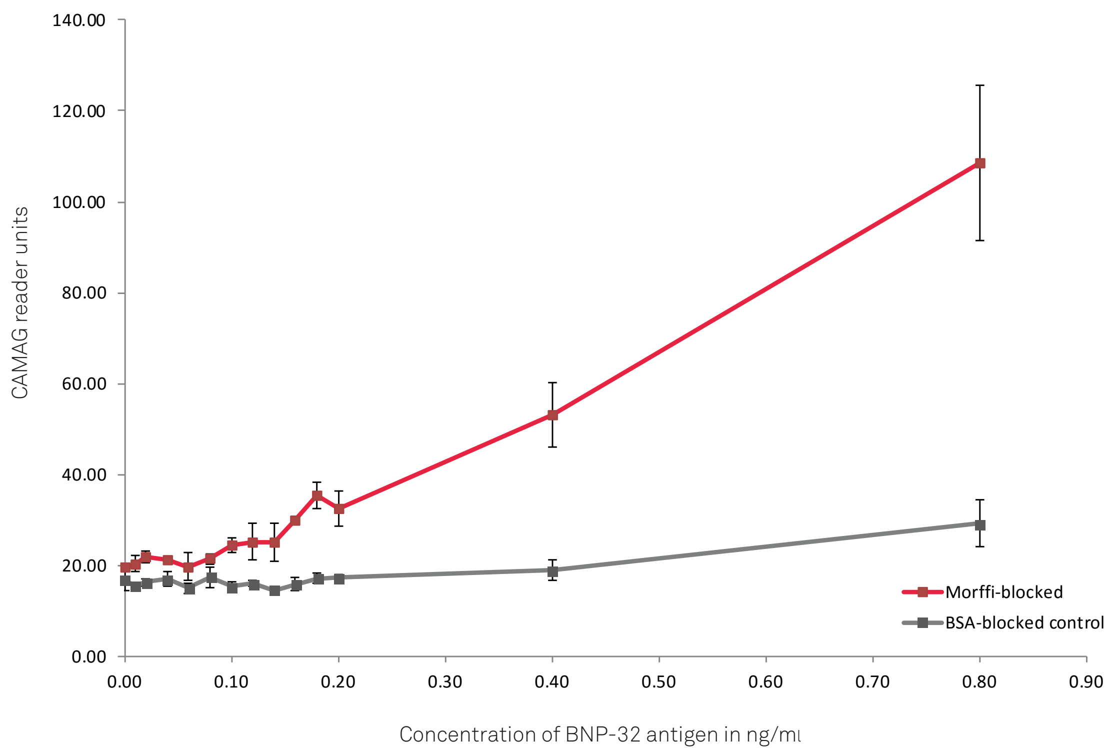

Figure 1: Mean signal intensity comparison for BNP-32 assay at low antigen concentrations.

Increased signal intensity

The results in Figure 2 demonstrate that the use of *Morffi* blocking technology leads to a significant increase in signal intensity compared to the BSA-blocked control for the BNP-32 LFI. This can be attributed to a reduction in non-specific binding and reporter cross-linking, leading to more efficient binding of the reporter to the antigen-capture antibody complex. At high antigen concentrations, this improved binding performance can be seen with the naked eye (Figure 3).

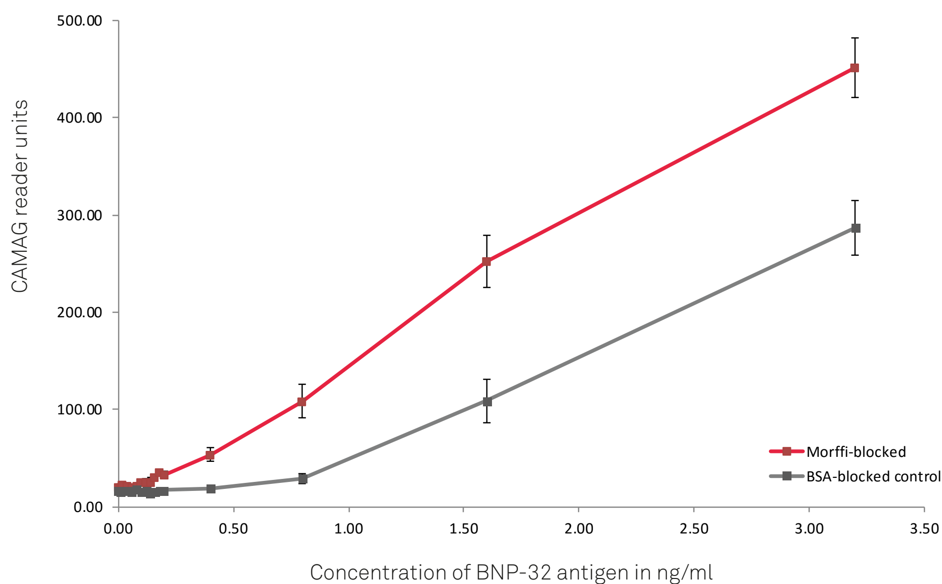

Figure 2: Mean signal intensity comparison for BNP-32 assay using conventional BSA-blocking and *Morffi* blocking technology.

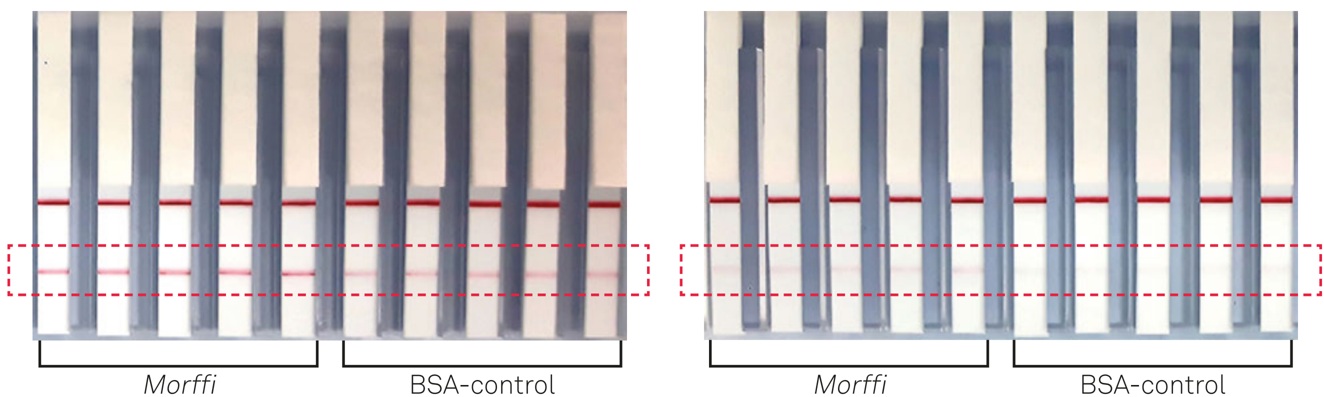

Figure 3: Visual comparison of LFI test strips using traditional BSA blocking and *Morffi* conjugate blocking technology at 3.2 ng/ml (left) and 1.6 ng/ml (right) antigen concentrations.

CONCLUSIONS

The results presented in this paper clearly demonstrate the feasibility of the *Morffi* conjugate blocking technology, offering a clear improvement in signal intensity over the BSA-blocked control for the BNP-32 assay system. This difference was visually discernible at high antigen concentrations, and provided a 10-fold enhancement in the limit of detection.

BBI has already used this technology to enhance the sensitivity of a number of other model assay systems - more than 30 conjugates have been tested to date - as well as several customers' tests routinely manufactured by BBI.

APPENDIX

Concentration of BNP-32 in ng/ml	0.00	0.01	0.02	0.04	0.06	0.08	0.10	0.12	0.14	0.16	0.18	0.20	0.40	0.80	1.60	3.20
BSA-blocked control mean reader signals	16.80	15.42	16.22	16.88	15.06	17.42	15.30	16.00	14.58	15.76	17.10	17.22	18.82	29.26	108.88	286.86
Morffi blocker mean reader signals	19.60	20.30	21.76	21.20	19.68	21.50	24.30	25.18	25.12	29.88	35.24	32.56	53.24	108.48	251.80	451.44

Conjugate condition	Concentration of BNP in ng/ml	CAMAG units			
		Test line signal	Mean test line	Standard deviation	2 x SE
Morffi-blocked	0.00	19.60	19.60	0.45	0.40
		20.00			
		20.10			
		19.10			
		19.20			
	0.01	20.90	20.30	2.00	1.79
		22.70			
		20.00			
		20.70			
		17.20			
	0.02	22.00	21.76	1.43	1.28
		21.80			
		20.00			
		23.90			
		21.10			
	0.04	21.20	21.20	0.64	0.57
		21.50			
		20.10			
		21.50			
		21.70			
	0.06	16.90	19.68	3.47	3.10
		23.40			
		22.50			
		20.20			
		15.40			
	0.08	20.30	21.50	1.28	1.15
		22.90			
		22.60			
		20.10			
		21.60			
	0.10	22.30	24.30	1.82	1.63
		25.20			
		22.60			
		26.60			
		24.80			
	0.12	27.70	25.18	4.50	4.02
		19.10			
		21.70			
		29.30			
		28.10			
	0.14	20.30	25.12	4.81	4.30
		28.50			
		25.70			
		20.20			
		30.90			
	0.16	31.10	29.88	0.90	0.81
		30.10			
		28.60			
		30.00			
		29.60			
	0.18	38.00	35.24	3.25	2.91
		35.10			
		29.90			
		37.70			
		35.50			
	0.20	34.40	32.56	4.34	3.88
		35.30			
		33.40			
		24.90			
		34.80			
	0.40	40.70	53.24	7.91	7.07
		51.00			
		55.70			
		57.70			
		61.10			
	0.80	110.40	108.48	19.09	17.07
		82.50			
		96.90			
		128.50			
		124.10			
	1.60	234.20	251.80	29.82	26.68
		229.00			
		263.20			
		233.30			
		299.30			
	3.20	492.30	451.44	34.46	30.82
		484.10			
		420.20			
		421.80			
		438.80			

Conjugate condition	Concentration of BNP in ng/ml	CAMAG units			
		Test line signal	Mean test line	Standard deviation	2 x SE
BSA-blocked control	0.00	15.50	16.80	2.76	2.47
		15.30			
		16.10			
		15.40			
		21.70			
	0.01	15.70	15.42	0.76	0.68
		15.20			
		14.90			
		16.60			
		14.70			
	0.02	15.30	16.22	1.00	0.90
		16.70			
		17.60			
		16.30			
		15.20			
	0.04	16.40	16.88	1.79	1.60
		19.10			
		15.30			
		15.20			
		18.40			
	0.06	16.10	15.06	1.25	1.11
		14.30			
		14.00			
		16.70			
		14.20			
	0.08	14.70	17.42	2.46	2.20
		16.30			
		19.50			
		20.50			
		16.10			
	0.10	16.20	15.30	1.05	0.94
		13.70			
		14.80			
		16.10			
		15.70			
	0.12	16.10	16.00	0.82	0.73
		16.40			
		15.90			
		16.90			
		14.70			
	0.14	15.20	14.58	0.59	0.53
		13.70			
		14.80			
		14.90			
		14.30			
	0.16	16.20	15.76	1.63	1.46
		18.40			
		15.00			
		14.30			
		14.90			
	0.18	16.40	17.10	1.26	1.13
		17.70			
		18.70			
		17.30			
		15.40			
	0.20	16.50	17.22	0.79	0.71
		16.90			
		17.80			
		18.30			
		16.60			
	0.40	20.70	18.82	2.50	2.23
		18.00			
		21.90			
		17.90			
		15.60			
	0.80	23.80	29.26	5.80	5.19
		22.50			
		35.40			
		33.50			
		31.10			
	1.60	79.30	108.88	25.07	22.43
		99.30			
		129.70			
		96.30			
		139.80			
	3.20	284.60	286.86	31.19	27.89
		323.00			
		245.50			
		311.10			
		270.10			

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New Product Development Leader with a degree in biochemistry. More than six years of experience developing a wide range of clinical lateral flow assays, as well as infectious disease and veterinary tests.

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Lateral Flow Product Marketing Manager with a degree in biomedical science and a masters degree in business administration (MBA). More than 13 years of experience in the gold nanoparticle and lateral flow development arena.

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