Performance Evaluation of Antibody and Antigen Conjugate Pair for Detection of Heroin Metabolite (6-MAM) in Lateral Flow Assay

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Introduction

6-monoacetylmorphine (6-MAM) is the primary and only opioid metabolite (Figure 1) specific to diacetylmorphine (heroin).[1] 6-MAM can be found from urine and blood samples of heroin users. Lateral flow remains one of the main analytical methods to detect heroin use.[2] Sensitivity of the assay is highly dependent on the optimal pairing of antibody and antigen conjugate. Medix Biochemica develops antibodies, antigens and other critical raw materials for in vitro diagnostics (IVD) tests. For the detection of 6-MAM, we identified hybridoma clone 12703 as the most sensitive candidate in the initial screening using a lateral flow assay. The objective of this study was to determine how different antigen conjugation ratios affect sensitivity of the lateral flow assay with clone 12703.

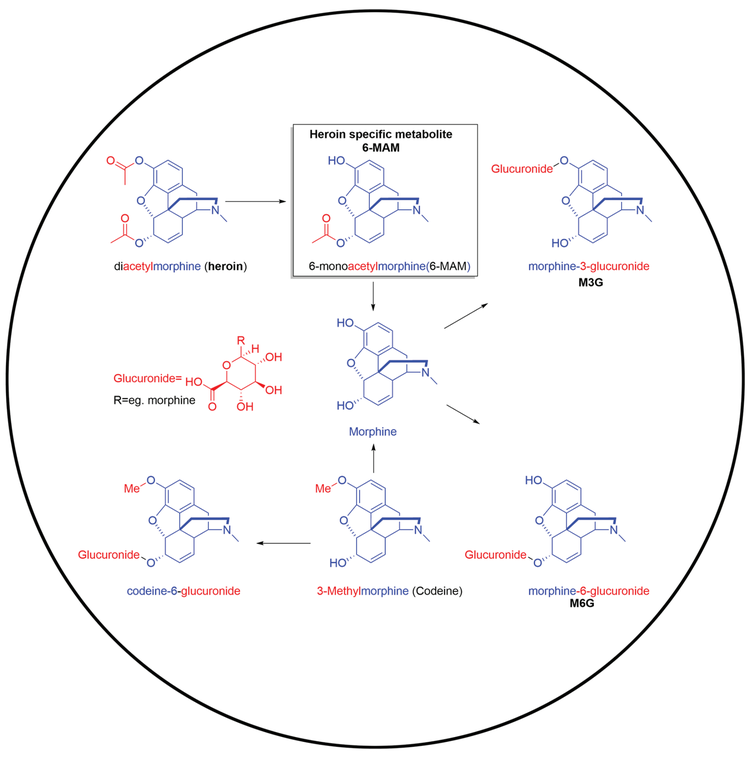

Figure 1. *Morphine/heroin metabolism in human.[1]*

Materials & Methods

We developed two antigen conjugates using different 6-MAM hapten:BSA ratios in conjugation reactions (20:1 and 23:1). For lateral flow assay, antibody 12703 was passively coated on 40 nm gold particles. Both antigen-BSA conjugate batches were coated onto nitrocellulose membrane (Sartorius CN140) at a fixed coating concentration of 0.5 mg/mL. The coated membranes were dried and stored desiccated until testing. The coated nitrocellulose membranes were laminated onto adhesive backing card along with other lateral flow pad materials. The laminated cards were cut into test sticks. Gold antibody-conjugate was applied to the test sticks and dried. Free 6-MAM was diluted in PBS/ 0.1% Tween buffer in concentrations ranging from 1.5625 to 50 ng/mL. PBS/ 0.1% Tween was used as a negative control. Each standard was tested in triplicate for each pairing condition. The intensity of the signals was quantified using a reader and results were plotted against the concentration of free 6-MAM to form standard curves.

Kinetics for 6-MAM mAbs were measured with biotinylated 6-MAM-BSA conjugate attached to streptavidin sensors using Octet RED96e bio-layer interferometry (BLi). Immunoreactivities against 6-MAM-BSA were measured with Europium(Eu)-based fluorescence immunoassay (FIA).

Results

We found that the conjugate with lower conjugation ratio (20:1, cat. 170031) resulted in greater sensitivity in lateral flow (Figure 2) when combined with 12703 compared to the one with higher conjugation ratio (23:1).

For manufacturing, the clone was turned into recombinant version (clone R12703, cat. 140041) and it was found to be comparable to original hybridoma clone in kinetic (Figure 3) and immunoreactivity (Figure 4) tests.

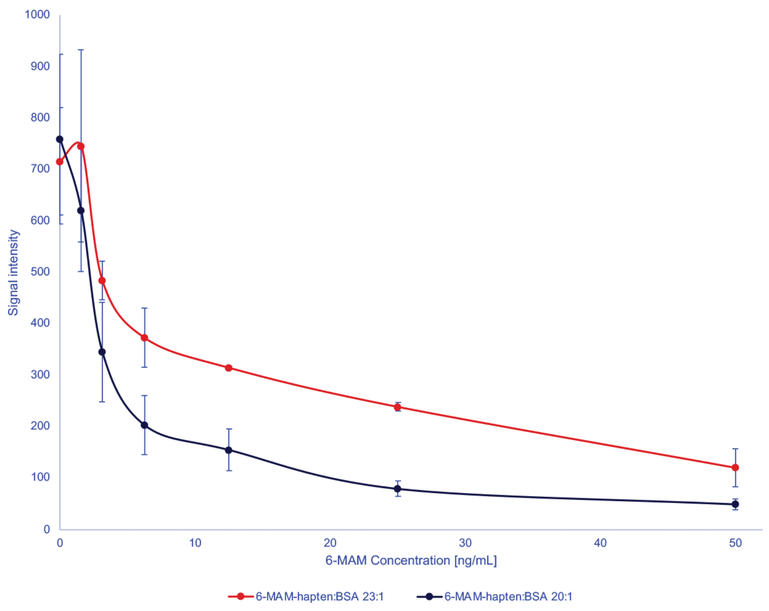

Figure 2. *Quantified signal intensities in Lateral flow.*

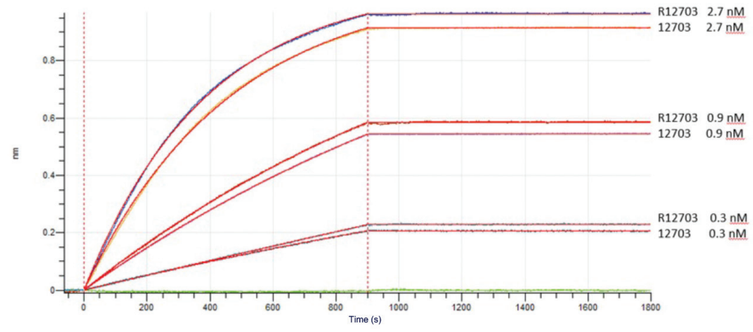

Figure 3. *Kinetic measurements of 6-MAM 12703 hybridoma and recombinant version. (R12703, k_a = 1.07 x 10^6 1/Ms, doesn't dissociate under test conditions).*

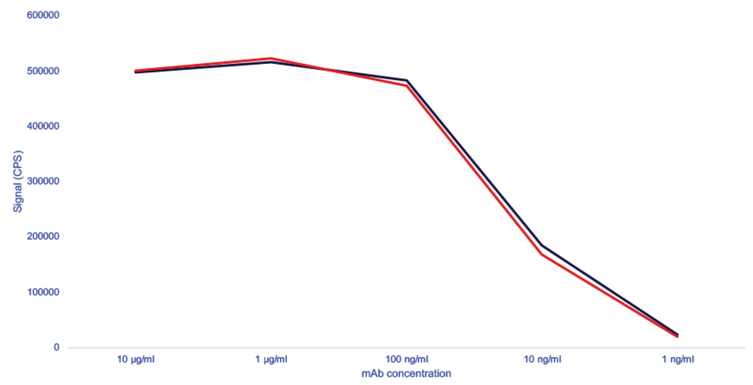

Figure 4. *Immunoreactivity of 12703 mAb vs. recombinant R12703 mAb in FIA format against Eu-labeled 6-MAM-BSA.*

Conclusion

We found that the 6-MAM-BSA conjugate with lower conjugation ratio (20:1 cat. 170031) resulted in greater sensitivity when combined with 12703 compared to the one with higher conjugation ratio. Recombinant version of 12703 was manufactured (R12703) and performance was demonstrated to be similar to hybridoma version in kinetic and immunoreactivity tests.

These results demonstrate that the developed 6-MAM mAb and antigen conjugate are promising raw materials for development of diagnostic immunoassays showing clearly distinguishable signal in concentrations (ng/ml) relevant to detection of 6-MAM from urine[3] and blood[4] in lateral flow.

References

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Acknowledgement

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