New Biotin Interference Free VITROS® MicroWell Technology In Next Gen Immunoassays On Ortho VITROS® 5600/XT 7600 Integrated Systems and VITROS 3600® and VITROS® ECi/ECiQ Immunodiagnostic Systems

P. Hosimer, S. Danielson, C. Thomas, H. Groth

Ortho Clinical Diagnostics, Rochester, NY

Introduction

Over-the-counter high dose biotin supplements can cause significant interference with immunoassays that utilize generic streptavidin-coated wells or particles.

An updated VITROS MicroWell Assay architecture is designed to be free of biotin interference. This technology has been used in all VITROS assays launched since 2015, including VITROS Anti-SARS-CoV-2 Total+, VITROS Anti-SARS-CoV-2 IgG+, VITROS TSH3*, VITROS PCT, VITROS HIV Combo, VITROS Insulin, VITROS C-Peptide, VITROS NT-proBNP II* and VITROS hs Troponin I*. VITROS MicroSlide and VITROS MicroTip technologies are not susceptible to biotin interference.

Background on Biotin-Streptavidin Architecture

Biotin-streptavidin architecture is commonly used in immunoassays due to the high binding affinity, which allows rapid and essentially irreversible binding to a capture surface. High endogenous biotin levels can suppress the assay's signal and produce a biased result. Negative bias will be observed in immunometric ("sandwich") assays, while positive bias will be observed in competitive assays.

Alternative surface immobilization methods are possible (i.e. adsorption or covalent attachment), but these can result in reduced antibody reactivity that may compromise analytical performance. The ideal assay design would preserve the benefits of indirect antibody attachment (biotin-streptavidin architecture), while avoiding biotin interference.

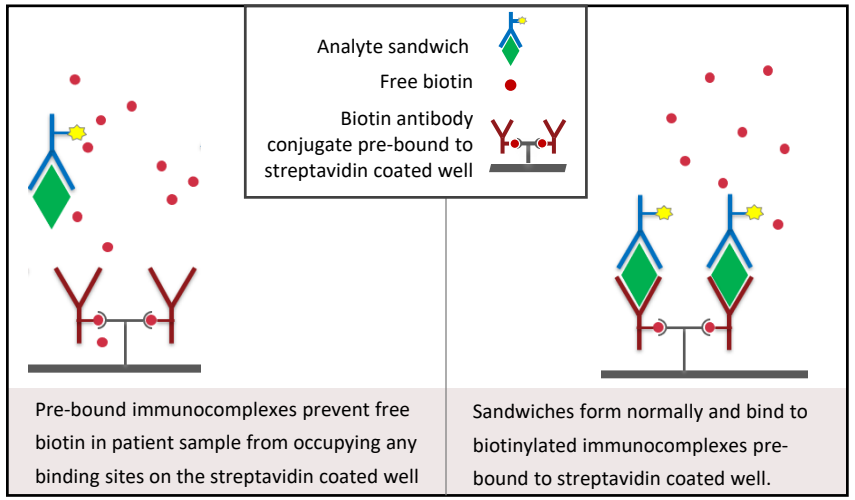
An Updated Assay Design for VITROS MicroWell Technology

The impact of biotin on assay performance depends on the assay format. Both VITROS MicroWell sandwich and competitive assay formats rely on binding immunocomplexes to a streptavidin-coated well.

Assay designs that use a streptavidin coated well simultaneously react to analyte present in the sample with a biotinylated antibody or an antibody/analyte complex (immunocomplex). If present, free biotin in the sample competes with the biotinylated immunocomplex for binding sites on the streptavidin-coated wells to falsely raise or lower results.

Since 2015, all new VITROS MicroWell assay designs have used a pre-bound assay architecture in which the antibody or antigen are immobilized to the well surface as part of the manufacturing process. This architecture is designed to preserve the benefits of biotin-streptavidin interaction, while being free from biotin interference.

Since the biotin-streptavidin binding is essentially irreversible, these pre-bound complexes are not displaced by free biotin in the sample, and no interference can occur. The presence or absence of biotin in the patient sample has no effect on the test.



Direct Coating of Antibody or Antigen		Pre-Bound Antibody or Antigen	
HIV	FT4	HIV Combo	Intact PTH II§
HBsAg	TT4	Vitamin D	NTproBNP II*
HCV	FT3	Insulin	hs Troponin*
aHBs	TT3	C-peptide	Anti-SARS CoV-2 Total+
aHBc	Anti-SARS CoV-2 IgG+	PCT	SARS CoV-2 Antigen#
		TSH3*	

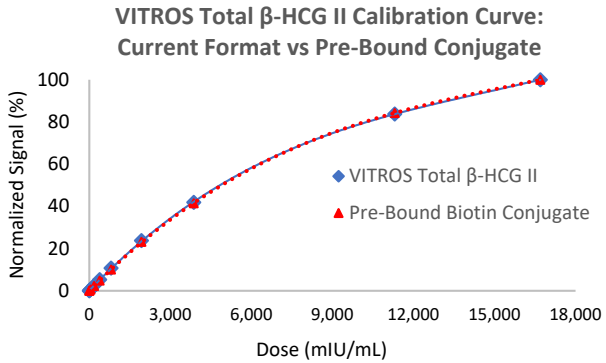
* Not currently available in US. Product availability in different countries may vary and be subject to local regulatory approval.

\+ EUA in US, CE marked
\# EUN in US, CE marked
§ In-development, not for sale.

Discussion

Ortho is converting VITROS MicroWell assays from the streptavidin-coated well format to the biotin-antibody conjugate pre-bound format. This is a relatively minor change, and assay performance has been shown to be remarkably equivalent to the current assays.

The graph below shows normalized signal data overlaid for Total b-HCG II and a modified version of the assay with the biotin-antibody conjugate pre-bound on the well. The slope of the calibration curve is virtually identical throughout the measuring range, and consequently assay performance is nearly identical.



Conclusion

The use of pre-bound biotin-antibody conjugates preserves the benefits of biotin-streptavidin architecture with a design that is free of biotin interference.

Biotin-free formulations are not required to prevent biotin interference.

CLSI EP07 and EP37 (2018) guidance recommends testing at 3510 ng/mL biotin concentration. Ortho has demonstrated performance free from biotin interference using the pre-bound format at these levels.

All newly developed VITROS assays launched since 2015 are designed to be free from biotin interference. In-market VITROS® MicroWell assays are being redeveloped to use a pre-bound assay design.

Presented at AACC Virtual Meeting, Dec. 13 – 17, 2020

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