Using sandwich ELISA to quantify free proprotein convertase subtillsin/kexin type 9 (PCSK9) in human serum

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Background

- Biosimilars are biological products that have similarities and no clinically meaningful differences from existing FDA-approved reference products.
- These biologics have the potential to reduce medication costs, expand patient access to important medications, and have led to treatment of many illnesses.
- Due to the demonstrated benefits of these drugs, the Biologics Price Competition (BPCI) Act was passed, creating a novel licensure pathway to promote biosimilar development.
- This pathway involves using pharmacological data to determine the biosimilarity between the proposed biosimilar product and the reference product, bypassing the need for expensive clinical studies.
- The utilization of the PD biomarker can be used alongside biomarker data from similar clinical studies to inform evidentiary standards for future pharmacodynamic biomarkers to be used for assessing similarity.
- In our study, we measured a potential PD biomarker and unbound human serum PCSK9 concentrations following the administration of Evolocumab or Alirocumab using a colorimetric assay.

Experimental Method

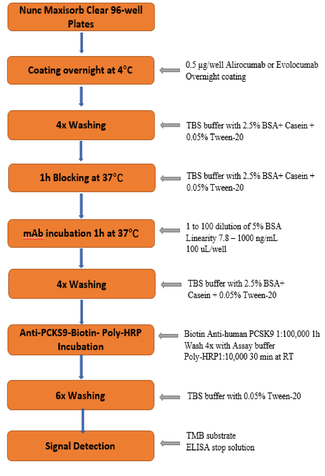
Figure 1: Free PCSK9 Assay Protocol

The utilization of a sandwich ELISA has been used in this project as a bioanalytical tool to measure the concentration of PCSK9 within human serum.

All volumes are 100 uL/well except for blocking buffer incubation and washing step, it will be 200 uL/well

Assay buffer: 2.5% BSA+Caesin+0.05% Tween-20

All incubations are on plate shaker at 300 rpm except for TMB and coating step



Experimental Method

Figure 2: Schematic of Sandwich ELISA

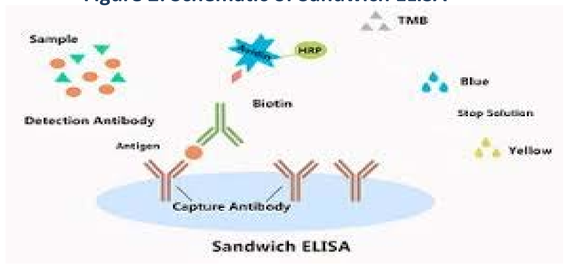


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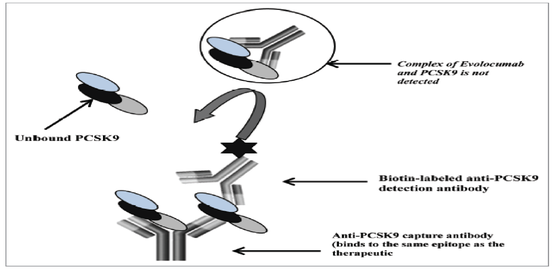
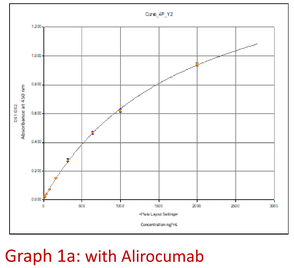
Figure 3: Schematic of Free PCSK9 Assay



Colbert, A., Umble-Romero, A., Prokop, S., Xu, R., Gibbs, J., & Pederson, S. (2014). Characterization of a quantitative method to measure free proprotein convertase subtilisin/kexin type 9 in human serum. *Mabs*, *6*(4), 1103-1113.

Figure 4: Linearity

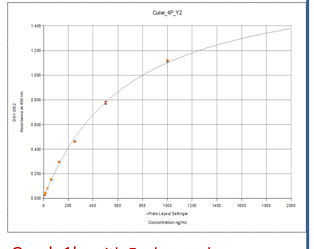


Graph 1a: with Alirocumab

Linearity: 20 to 2000 ng/mL

Coefficient of Correlation (r^2): 1.0

Signal to Noise at LLOQ: > 10



Graph 1b: with Evolocumab

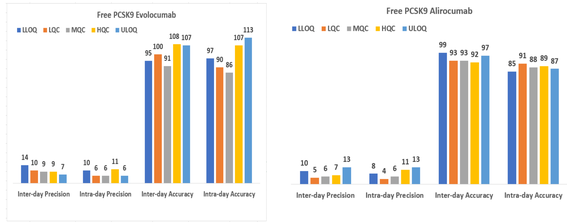
Linearity: 7.8 to 1000 ng/mL

Coefficient of Correlation (r^2): 1.0

Signal to Noise at LLOQ: > 10

Results & Discussion

Figure 5: Precision and Accuracy



Acceptance criteria

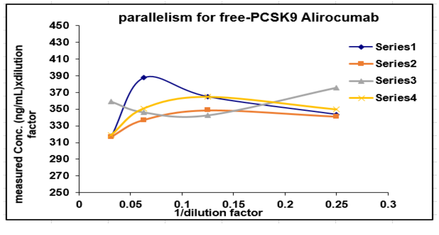
With-in run accuracy should be ± 20% of nominal concentration except for LLOQ and ULOQ , which should be within ±25% of nominal concentration
With-in run precision should be ± 20% CV except for LLOQ and ULOQ, which should be within ±25 CV

Figure 6: Bench Top and Freeze Thaw Stability

Acceptance criteria

Data should be ± 20% of nominal concentration except for LLOQ and ULOQ , which should be within ±25% of nominal concentration

Figure 7: Current Free PCSK9 Alirocumab Clinical Data



Acceptance criteria

For a well-developed ligand-binding assay, the calibration curve concentrations should be parallel to support the assumption that the antibody-binding characteristics are similar enough to allow the determination of analyte levels in the diluted samples.

Conclusion

- Two separate sandwich ligand binding assay methods were developed to measure unbound PCSK9 concentrations in human serum using bovine serum albumin as a surrogate matrix.
- The methods showed linearity from 7.8 ng/mL to 1000 ng/mL for Evolocumab and 20 ng/mL to 2000 ng/mL for Alirocumab with no effect on the matrix.
- Both the methods were validated as per the FDA bioanalytical method validation guidance.
- This free PCSK9 quantification provided insight into how biomarkers, like PCSK9, can be used to assess the pharmacological effect of the drugs.
- Prior to the study sample analysis, parallelism experiment was conducted to evaluate the effect of serum concentration on ruggedness of the method and concluded that there was no effect.
- The long-term stability of the stored QC (LQC and HQC at -80^{O}C) was performed for 66 days.

Acknowledgement

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Disclaimer

The findings and conclusions in this article have not been formally disseminated by the Food and Drug Administration and should not be considered to represent any agency determination or policy.

References

Colbert, A., Umble-Romero, A., Prokop, S., Xu, R., Gibbs, J., & Pederson, S. (2014). Characterization of a quantitative method to measure free proprotein convertase subtilisin/kexin type 9 in human serum. *Mabs*, *6*(4), 1103-1113. https://doi.org/10.4161/mabs.28719

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