# Implementation of Holistic Glycopeptide PASEF-DDA Data In Diagnostics

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## Introduction

- Shotgun glycoproteomics in blood plasma offers unique possibilities for clinical applications in multiple genetic and acquired human diseases
- Advantages of holistic PASEF-DDA data acquisition for clinical applications:
  - Single data file can be used for both biomarker research and application
  - All possible MS1 and MS/MS data is generated for each sample to enable retrospective analysis of novel biomarkers
  - Different disease biomarkers can be evaluated by simply exchanging the glycosylation site targets used for targeted feature extraction
- Targeted feature extraction enables efficient computational data processing
- Extracted glycopeptide features are used to infer site-specific glycosylation profiles which are translated into lucid clinical test reports.

## Materials and Methods

- Glycopeptides are enriched from tryptic blood plasma digests and analyzed by C18RP liquid chromatography with online parallel accumulation serial fragmentation data dependent acquisition (PASEF-DDA) on a Bruker Daltonics timsTOF Pro instrument.
- A selection of glycosylation site reference data is used as input for targeted feature extraction in DataAnalysis 5.3
- Glycopeptide feature intensities are converted into site-specific glycan stoichiometry profiles and subsequent relative glycan class information
- Patient sample data and batch controls are compared to control reference information to find statistically significant changes in glycobiology
- Metadata, QC information, statistical analysis results, and site-specific glycosylation profiles are summarized and visualized in the final diagnostic test report.

## Diagnostic Workflow

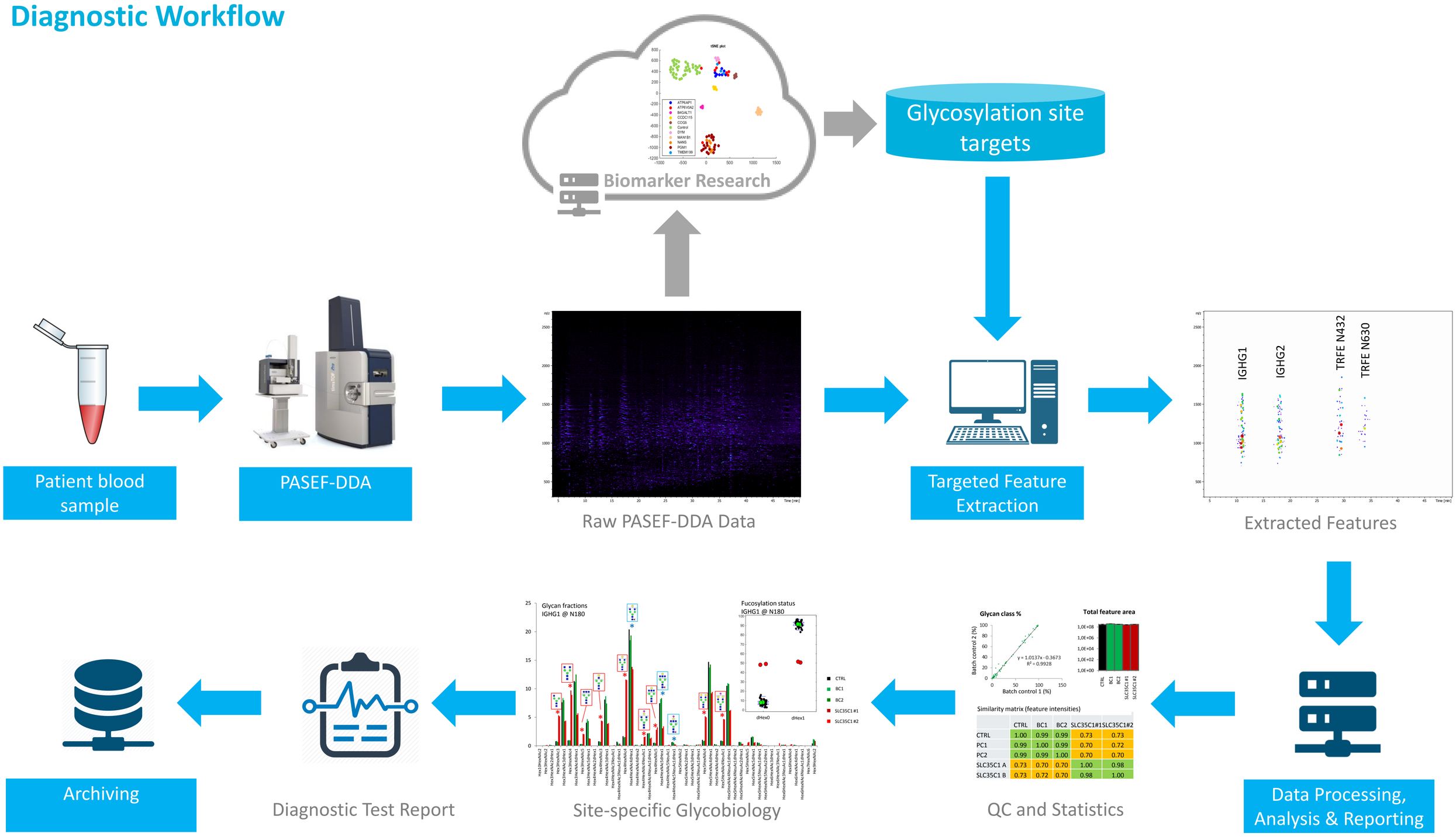


**Figure 1: Diagnostic glycopeptide workflow.** In this proof-of-principle case example a batch of patient samples and two batch control samples (BC1 and BC2; n=5 pooled healthy control samples) were prepared and analyzed by C18RP PASEF-DDA. Replicates of a patient with GDP-fucose transporter deficiency (SLC35C1) and the two BC samples were compared to reference data from n=40 healthy individuals. Quality control statistics and visualisations at all hierarchical data levels enable intuitive evaluation of the data quality. The site-specific glycosylation profile for N180 of IGHG1_HUMAN showed a marked decrease in fucosylated glycan species in the patient samples. Reference glycan images are shown for statistically significant changes to aid interpretation. A marked overall reduction of ~-40% fucosylation at IGHG1_HUMAN glycans was observed in this patient compared to healthy individuals.

## Conclusions and Future Perspectives

- Holistic PASEF-DDA data is of sufficient quality for use in both biomarker research and diagnostics.
- Glycopeptide workflow for diagnosis of congenital disorders of glycosylation will be implemented in clinical care in 2021
- Use of real-time glycopeptide data processing and reporting by GPU-parallelisation will be explored for next generation workflows

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