

Improving Standard N-Glycan Sample Preparation with Manual Automation Using Microchromatography to Improve Efficiency, Accuracy, and Reproducibility

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OVERVIEW

Purpose

The search for more efficient tools that streamline the sample preparation process for characterization of N-Glycans continues to be at the forefront in the field of glycomics. The goal is to more effectively streamline the sample preparation and data analysis to allow for a deeper understanding of the multiple structures and functions that result from glycosylation. Adding sugars to proteins in the process of forming glycoproteins can be complex. Abnormal glycosylation, congenital disorders of glycosylation (CDG), can occur and be linked to a number of diseases. Better data and documentation of this structure and function relationship can assist with understanding how these diseases occur and thus further the development of disease biomarkers. Monitoring bioprocessing of monoclonal antibodies (mAb) is another area where product glycosylation is important. Eliminating manufacturing variability from batch-to-batch is critical, but the traditional laboratory sample preparation approach can often take too long.

Traditionally, sample preparation for N-Glycans takes more than three days and often involves specific laboratory technique, not including the time for sample analysis. The transition to manual automation tools combined with microchromatography reduces sample preparation to less than 2.5 hours, while providing the added confidence that N-Glycan sample preparation and the sample data are reliable. Human Immunoglobulin G (hIgG) was mixed with bovine fetuin prior to microchromatography purification and subsequent sample preparation, labeling and analysis using HPLC with fluorescence detection. Results indicate that manual automation tools show improved accuracy and reproducibility between samples while allowing for more effective and more efficient characterization of N-Glycans to be performed.

Method

N-Glycan sample preparation was performed using manual automation with the Gilson TOOLKITS. Percent area was calculated for each of the six major free glycan peaks from multiple injections (n=3) run from the Gilson GX-271 Analytical HPLC System with Jasco FP-2020 Plus Intelligent Fluorescence Detector. Time savings, accuracy compared to a independent standard, and reproducibility between samples was calculated.

Results

Sample preparation of N-Glycans using manual automation with the Gilson TOOLKITS in combination with the ProZyme GlykoPrep™ Kit allows for 1-24 samples to be prepared in a nearly error-free environment in order to streamline from days with the well-known traditional method to just under 2.5 hours with a more effective and more efficient sample preparation process with %CV values less than 3.1% for any of the six major free N-Glycan peaks.

EXPERIMENTAL

This application discusses the benefits of manual automation for N-Glycan sample preparation using the Gilson SD TOOLKIT (Single channel Diagnostic) and Gilson MD TOOLKIT (Multichannel Diagnostic) with the GlykoPrep protocol to provide automatic sample preparation tracking and electronic protocol display using the TRACKMAN™. In combination with the ergonomic design of the Gilson PIPETMAN™ pipettes, accurate sample loading of the AssayMAP cartridges throughout the GlykoPrep protocol is further enabled with the use of the uniquely designed gradation ridges of the Gilson PIPETMAN pipette tips. The GlykoPrep protocol uses innovative AssayMAP® cartridges, a microliter-scale analytical bind/elute chromatography, to perform sample preparation in a matter of 2-3 hours versus days.

The historical bottleneck of glycoanalysis is drastically reduced to allow for faster characterization. AssayMAP technology scales standard chromatography practices to the microliter range, enabling high-throughput preparation and analysis of samples with known, workable resin chemistry in microchromatography cartridges. Bind-and-elute chromatography methods are used to both purify and quantitate samples.

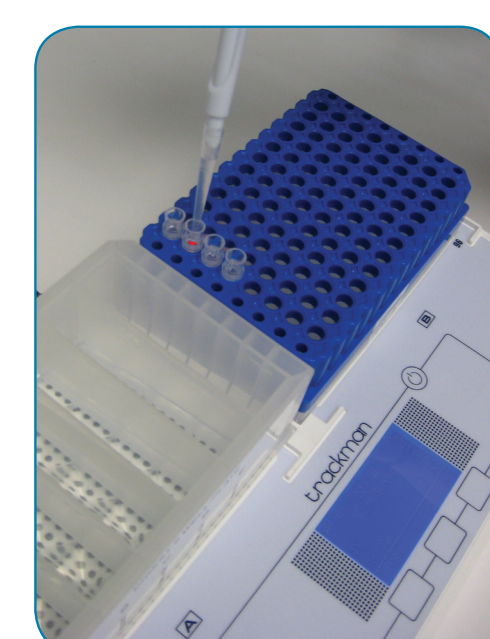


Figure 1. Gilson TOOLKIT N-GLYCAN Using the ProZyme GlykoPrep™ Kit

METHOD

Samples and Solvents

- Fetuin, from Fetal Calf Serum (Sigma, P/N F3385)
- IgG from Human Serum, Technical Grade, >80% SDS-PAGE (Sigma, P/N 18640)
- NanoPure Water
- HPLC Grade Acetonitrile
- Mobile Phase Buffer Preparation
 - Formic Acid, 99+% - (Acros Organics, P/N 270480010)
 - Ammonium Hydroxide – HPLC Grade (Fluka, P/N 17837)

Apparatus

- Gilson TRACKMAN with GlykoPrep Protocol
- Gilson PIPETMAN M single & PIPETMAN Neo Multichannel Pipettes
- Gilson PIPETMAN Tips
- Gilson GX-271 Liquid Handler with 402 Dual w/ Tee Dilutor
 - 10 mL syringe & 100 µL syringe
 - 5 SC pump heads
- Gilson 811D Dynamic Mixer
 - 1.5 mL Analytical Mixing Chamber
- Gilson 805 Manometric Module
- Column Heater – Set to 50°C
- Jasco FP-2020 Plus Intelligent Fluorescence Detector
- ProZyme GlycoSep™ N-Plus Column (P/N GKI-4730) and Guard Column

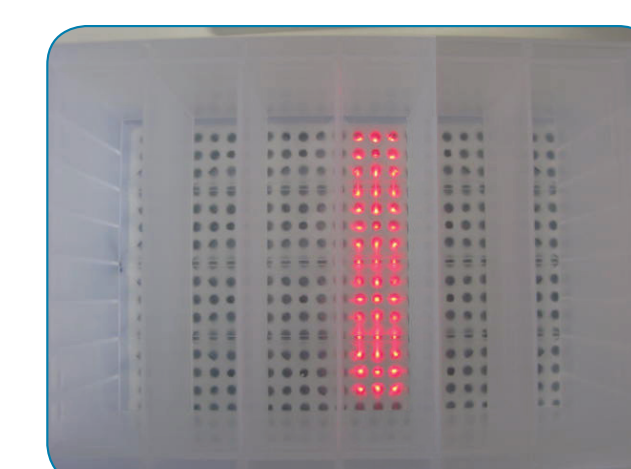


Figure 3. TRACKMAN™ Illuminating the Solvent Plate and AssayMAP Cartridges



Protocols

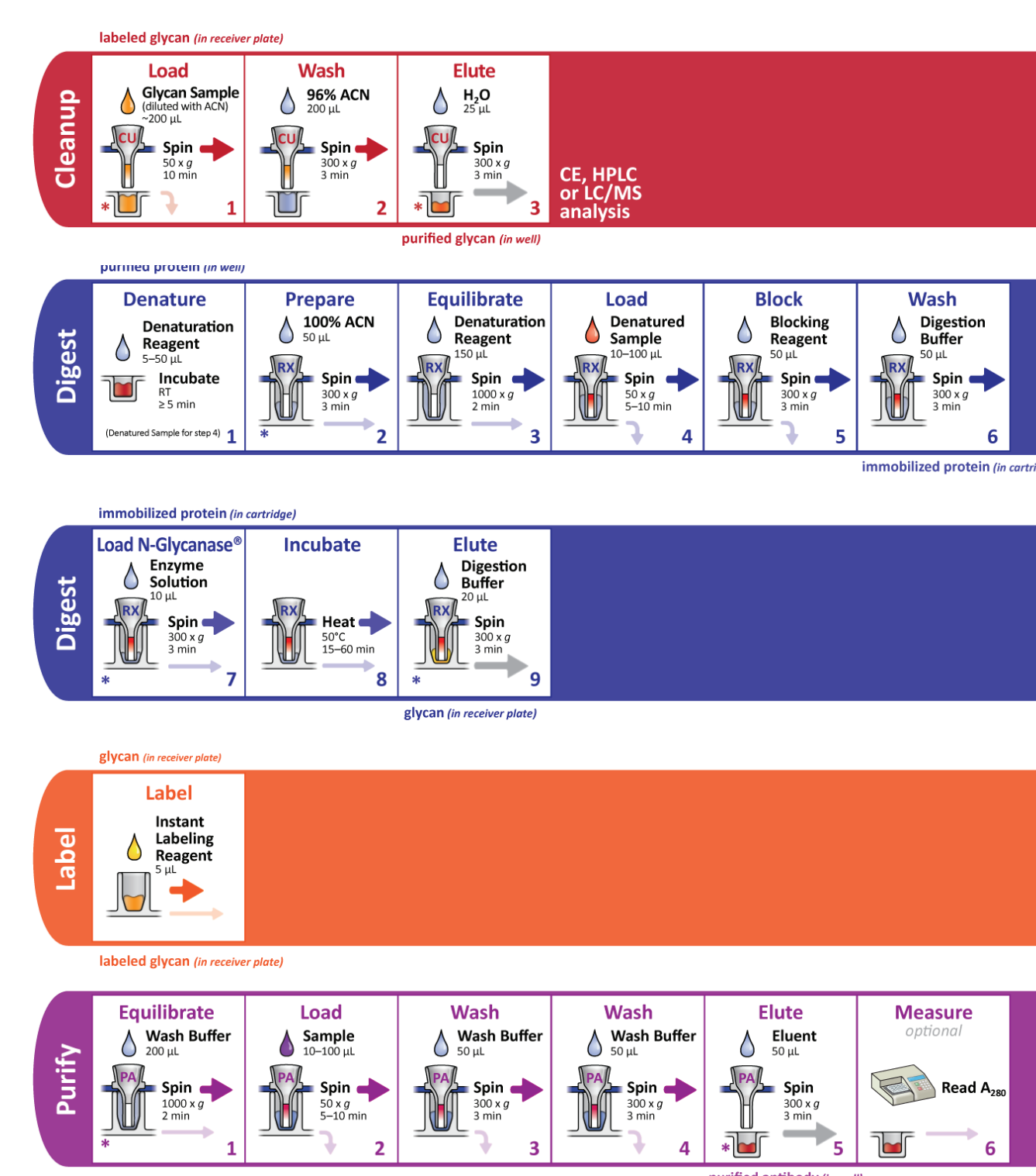


Figure 4. Rapid N-Glycan Preparation ProZyme GlykoPrep™ Protocol

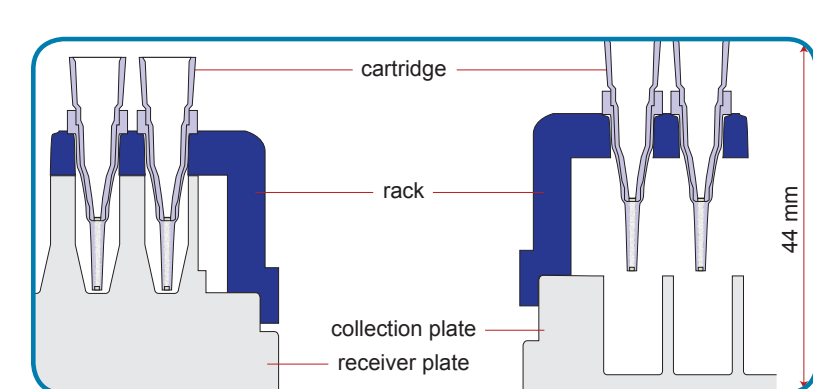


Figure 2. AssayMAP™ Technology Used in the GlykoPrep™ Kit



RESULTS AND CONCLUSION

Rapid N-Glycan sample preparation of the GlykoPrep Protocol from ProZyme was made even more efficient, consistent, and error-free with the use of the Gilson SD TOOLKIT and Gilson MD TOOLKITS. The TRACKMAN GlykoPrep protocol allowed for accurate pipetting and effective tracking throughout the multi-step protocol using text screens and lighted wells. Chromatographic results showcased accurate sample purification with Protein A and preparation when compared with the independent standard. The use of the Gilson TOOLKITS eliminated the hassle of manually checking after each step in the protocol and further increased efficiency by letting the TRACKMAN keep track of which cartridges were loaded and processed. This saved valuable time and decreased the total time to perform the GlykoPrep protocol.

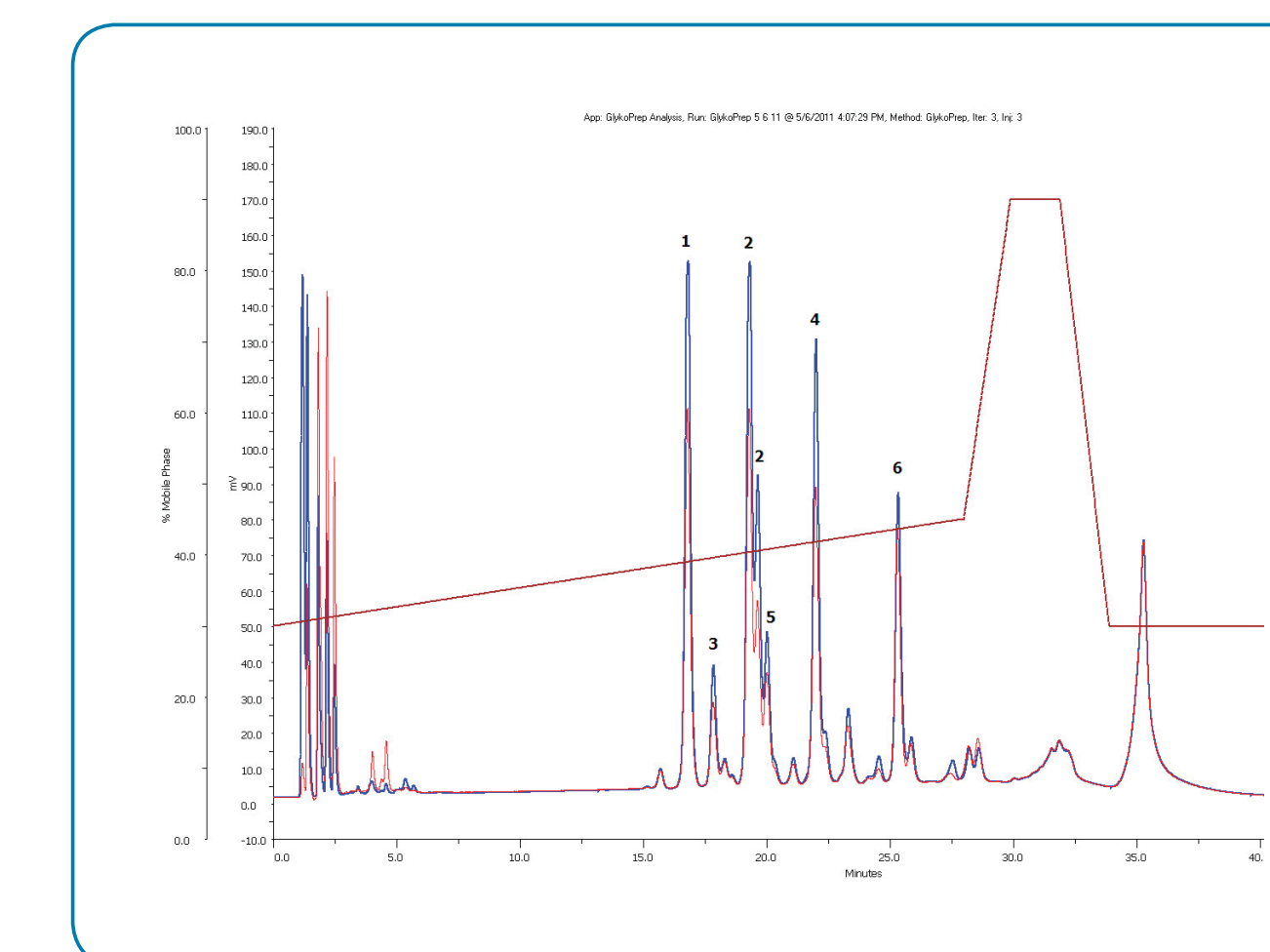


Figure 5. Independent Standard Injection (Blue Trace) Overlaid with Mixed Impure hlgG + Fetuin Sample Injection (Red Trace)

Day-to-day variation in results can be reduced with the use of the Gilson PIPETMAN disposable tips on either the PIPETMAN M or PIPETMAN Neo pipettes. The TRACKMAN GlykoPrep Protocol allows for ease in transitioning the number of samples that are run per day; from 1 to 4 and 8, 16, 24 samples. As shown by the consistency in results (See Table 2), the gradation line on the DIAMOND tips in combination with the unique features of the PIPETMAN pipettes enabled consistency in pipetting technique for a manual automated method. Analysis via HPLC with fluorescence detection can be very consistent and provide reliable results as seen by the retention time reproducibility data shown in Table 3.

Peak Number	Peak Name
1	G0F (NGA2F)
2	G1F (NA2G1F) isomers
3	G0FB (NGA2FB)
4	G2F (NA2F)
5	G1FB (NA2F1FB)
6	G2F51 (A1F)

Table 1. Identification of Peak Names from hlgG Chromatogram

Peak Number	% Mean Area	Standard Deviation	% CV
1	21.260	0.310	1.458
2	34.697	0.186	0.538
3	4.733	0.133	2.820
4	17.221	0.308	1.793
5	9.076	0.252	2.786
6	13.101	0.397	3.056

Table 2. TRACKMAN GlykoPrep Protocol Peak Area Reproducibility (n = 3)

Peak Number	Mean Retention Time (min)	Standard Deviation	% CV
1	17.180	0.064	0.372
2a	19.681	0.060	0.304
2b	19.979	0.065	0.325
3	18.177	0.066	0.363
4	22.332	0.065	0.291
5	20.354	0.065	0.319
6	25.569	0.036	0.140

Table 3. TRACKMAN GlykoPrep Protocol Peak Retention Time Reproducibility (n = 3)

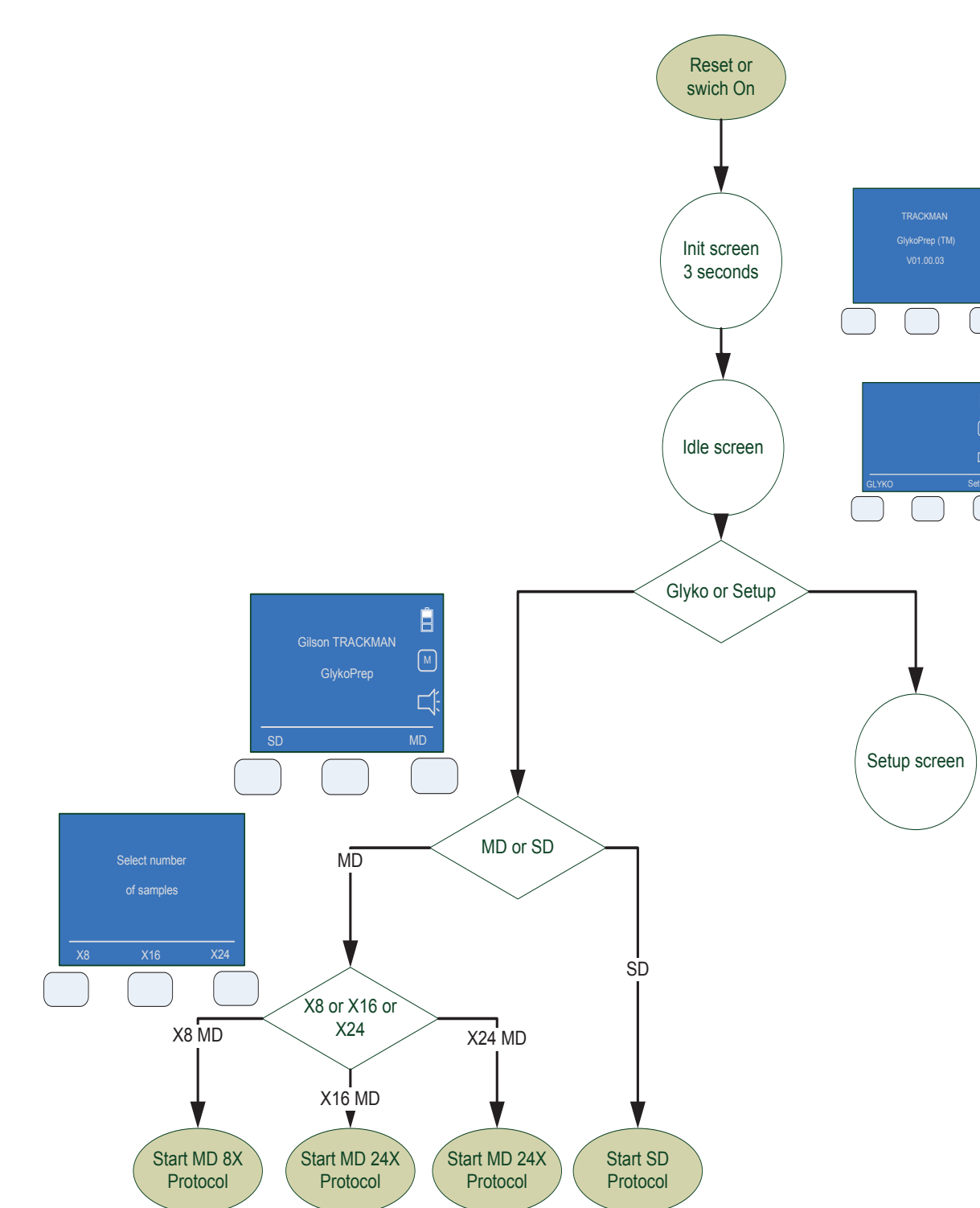


Figure 5. TRACKMAN™ GlykoPrep Protocol Map