Rapid Identity Assays for mAb Development, Production Control and Release

Anja Resemann1, Waltraud Evers1, Yue Ju1, Guillaume Tremintin1, Detlev Suckau

1 Bruker Corporation, Bremen, Germany
2 Bruker Corporation, Billerica, MA, USA
3 Bruker Corporation, San Jose, CA, USA

see (Bruker) in workflows. according to cosine similarity scores.

Methods

Several antibodies were used in this study either as medicinal formulations (NIST mAb, Adalimumab, Trastuzumab, Pantumumab, Cetuximab and Natalizumab).

For clone selection they were digested using IdeS (Genovis), diluted into DMAP or sinapinic acid MALDI matrix and the 2+ charge state was used for glycan profiling in linear mode MALDI-MS analysis.

For rapid release identity testing they were incubated in 50% 13C glucose, 50 mM DTT (5 min, 50°C) digested using trypsin/Lys-C (Promega), 5 min after digestion with digestion buffer (Fig. 8).

Samples were analyzed by MALDI on an autoflex max (Bruker) in reflector mode using CCA matrix. Automatically acquired spectra were processed in BioPharma Compass 3.0 (Bruker). Antibody identity was confirmed based on the peptide profile similarity while rapid glycan profiling was based on Fc-linked glycan profiling.

Figure 1: Pass/fail results of the analysis are mapped directly to the position of samples on the MALDI plate. For research use only. Not for use in diagnostic procedures.

Figure 2: Samples can be compared to reference data: automatically (top: cosine similarity score of NIST vs. NIST glycan profiles – see Fig. 3) and visually (bottom: butterfly plot of cetuximab vs. NISTmAb tryptic digest – see Fig. 4).

Figure 3: NISTmAb Fc- glycoproteins from matrix ESI-UHR-QTOF (top, deconvoluted, Mr mono) and from autoflex max MALDI peptide data. Inserts show the peptide data view with the traffic light reporting fields as explained in Fig. 2, top. Detailed match report bottom.

Figure 4: Rapid ID Testing with a NISTmAb test profile against another NISTmAb rapid ID successfully confirmed NISTmAb. Matches match cetuximab and trastuzumab against according to cosine similarity scores. ID not confirmed / Mass accuracy within MS Tol.

Figure 5: Fifteen min Rapid Identity Testing Chemistry

BioPharma Compass 3.0 software supports the routine analysis of biopharmaceuticals, both with LC-ESI and LC-free MALDI workflows. Here we discuss applications of MALDI to high throughput clone selection and to the rapid identification of mAb samples, e.g., for Fill & Finish Operations, within 20 min from intact mAb sample to identification report.

Introduction

During biopharmaceutical development (e.g., clone selection) and production (rapid release identity testing) there is a requirement for fast analysis return times to accelerate decision making and reduce costs. We utilized rapid protein digest methods and integrated MALDI-TOF sample analysis with a software workflow to compare measurements against a reference attribute profile. This comparison was used in clone selection workflows to screen glycan profiles in intact Fc-domains and to provide antibody identities rapidly, based on differentially abundant peptides in peptide mass fingerprints.

These protocols were developed to achieve analysis return times from intact antibody samples to automatic identity confirmation based on trypsin/Lys-C digests of 15 min and for Fc-glycosylation profiling within 1/2 hour.

Digest and sample preparation time of IdeS digestion (Bruker) was 1/2 hr. Major glycans such as G0F, G1F, G2F and G3F were assayed by direct profiling of the Fc-domain of monoclonal antibodies. Spectra acquisition and processing were completed in less than 10 sec/sample. Different attributes such as the mass of the glycan profile with a QTOF reference profile (Fig. 3) with a certain score (Fig. 4, top) or the test for G3F as being the base peak glycan were reported in the software providing multiple data points rapidly to decide which clones to select for further rounds of screening.

Conclusions

- BioPharma Compass allows to take advantage of MALDI MS spectra through comparison of target/reference profiles with test samples
- Typical applications supported: screening in early development, such as clone selection
- Rapid Release ID Testing during Fill & Finish operations in approx. 12 min
- QC of incoming goods such as Tween20 vs Tween 80 differentiation. (not shown)
- 2-AB glycan profiling (LC-free, not shown)

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