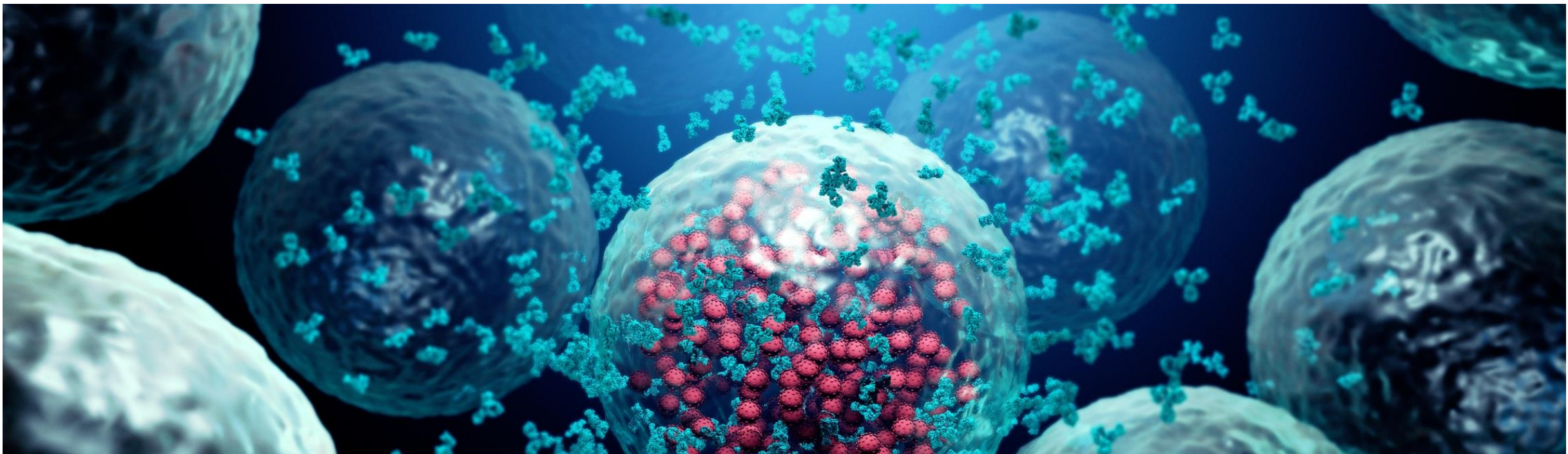


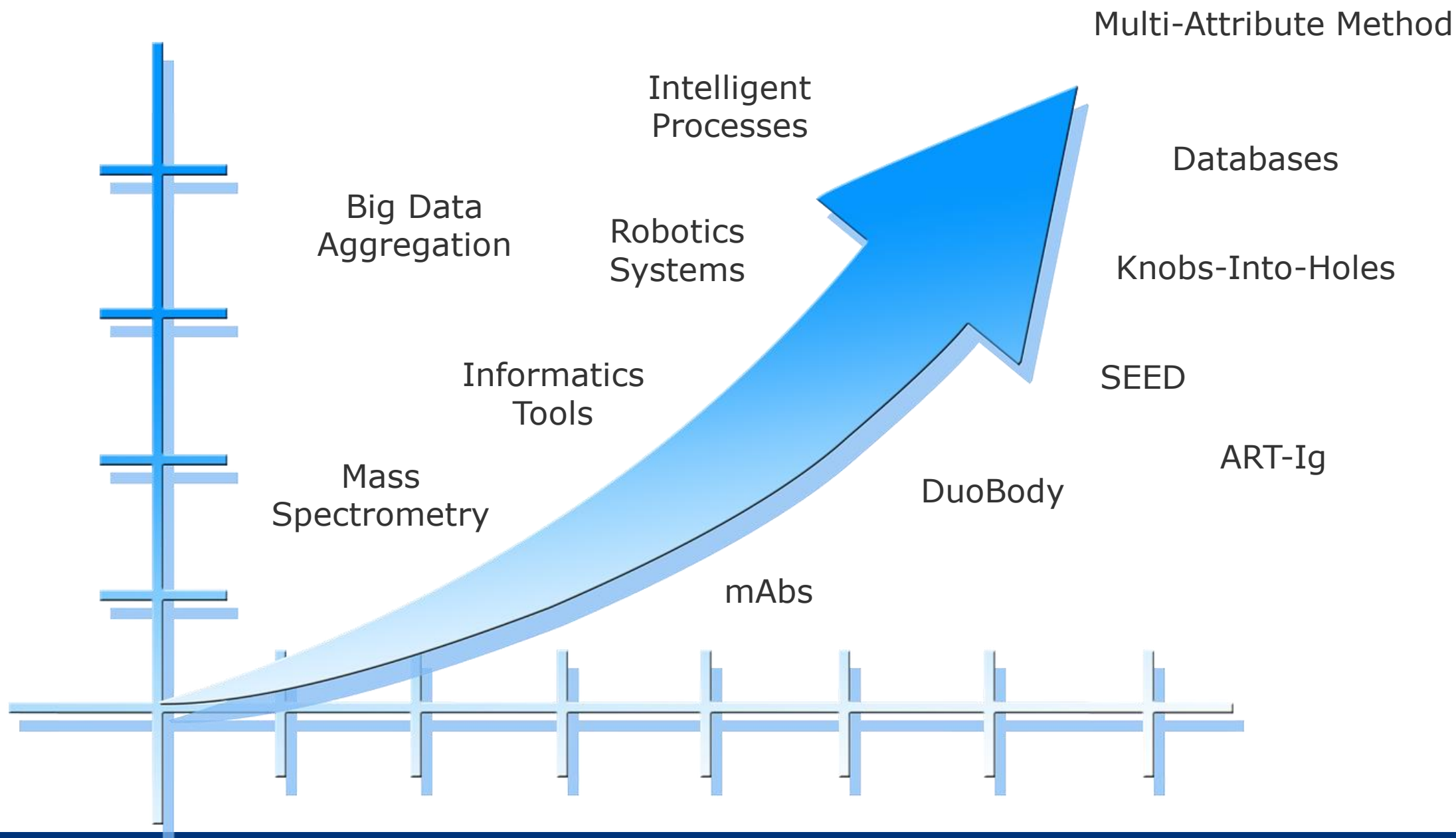


Development of Automated Workflows for Analysis and Reporting of Peptide Mapping Data

Michael E. Pettit, PhD
Janssen Research & Development, LLC
BioTherapeutic Development & Supply | Analytical Development
28 September 2022

Antibodies destroy an infected cell

Trending for End-to-End Automation



Overview of Discussion Topics

Automating analysis of Targeted Peptide Mapping Data

Modification	Reference RM1 N/A [%]
Antibody 1 N-term Q Gln->pyro-Glu	81.1
Antibody 1 N-term Q Gln->pyro-Glu	99.3
Antibody 1 M34 Oxidation	0.3
Antibody 1 M103 Oxidation	1.7
Antibody 1 M108 Oxidation	2.2
Antibody 1 W32 Oxidation	0.7
Antibody 1 N55/N59 Deamidation	4.1
Antibody 1 Isomerization	0.5
Antibody 1 Isomerization	0.9
Antibody 1 R61 SV [Arg->Lys]	1.0
Antibody 1 N392/N397 N386/N391 Deamidation	4.5

1. Creating Automated Workflows in Genedata

I. Peak Mask vs XIC Workflows

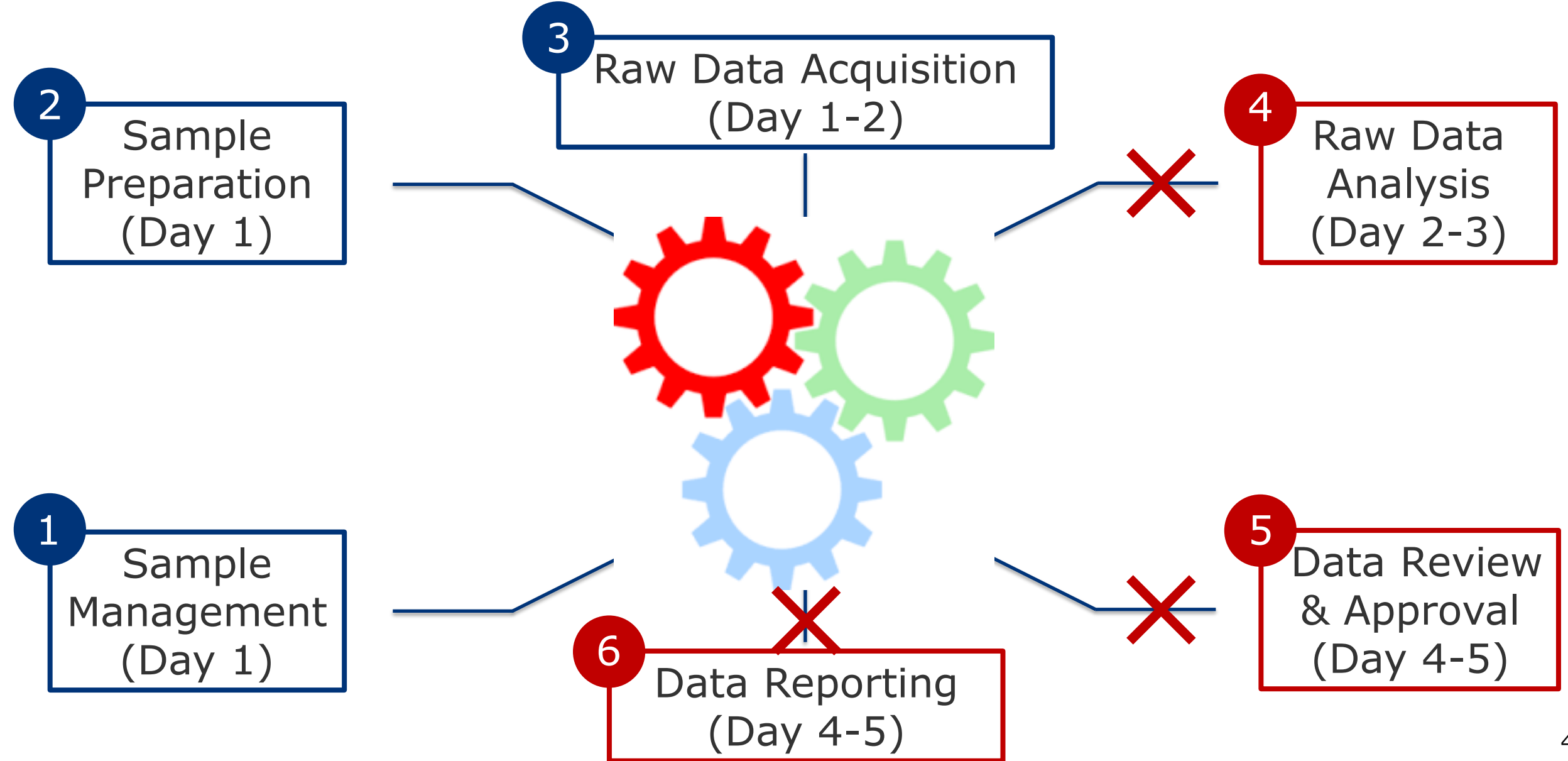
II. Custom plug-in development

2. Evaluating Automation vs Manual Results

I. Compare to historical data (N=24)

II. System Suitability

Manual Analysis & Reporting is Current Bottleneck



Not Just a Bottleneck



Manual, labor intensive, & require Analyst + SME

Auto-generated & auto-formatted

Contains product & sample specific info

Populated with high-confidence calculations

System suitability test results

allow **manual** data
analysis & reporting into
end-to-end automation

Product-Specific Template for Automated Analysis & Reporting

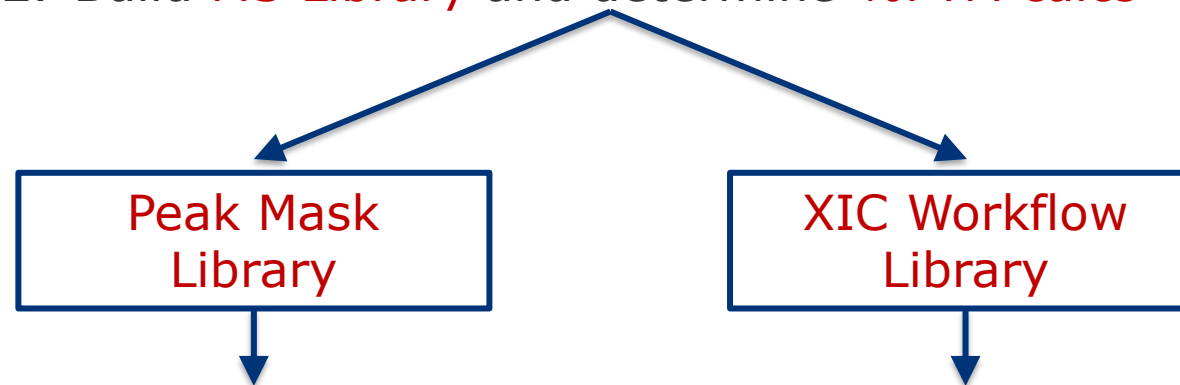
Automating analysis of Targeted Peptide Mapping Data

Modification	Reference RM1 N/A [%]
Antibody 1 N-term Q Gln->pyro-Glu	81.1
Antibody 1 N-term Q Gln->pyro-Glu	99.3
Antibody 1 M34 Oxidation	0.3
Antibody 1 M103 Oxidation	1.7
Antibody 1 M108 Oxidation	2.2
Antibody 1 W32 Oxidation	0.7
Antibody 1 N55/N59 Deamidation	4.1
Antibody 1 Isomerization	0.5
Antibody 1 Isomerization	0.9
Antibody 1 R61 SV [Arg->Lys]	1.0
Antibody 1 N392/N397 N386/N391 Deamidation	4.5

1. Determine which PTMs to monitor

Leverage LC-MS/MS Peptide Mapping Data from Forced Degradation Structure Function Studies

2. Build MS Library and determine %PTM calcs



3. Template for Automated Data Analysis & Reporting

PTM Monitoring by Peak Mask vs XIC Workflow (Genedata)

	Peak Mask Workflow	XIC Workflow
Method Type	Targeted MS1	Targeted MS1
MS1 Library	Sequence, RT, mass values	Sequence, RT, <i>m/z</i> values of charge-states & isotopes
Peak Mask?	Requires Peak Mask	Does not require Peak Mask
LC-MS Peak Detection	All chromatograms are self-aligned , then aligned to Peak Mask	Individual chromatograms are compared to RTs from MS1 Library
Library Searching	Data that fit Peak Mask are further processed	Data that fit isotopic pattern are further processed

Peak Mask Workflow: MS1 Library + Peak Mask

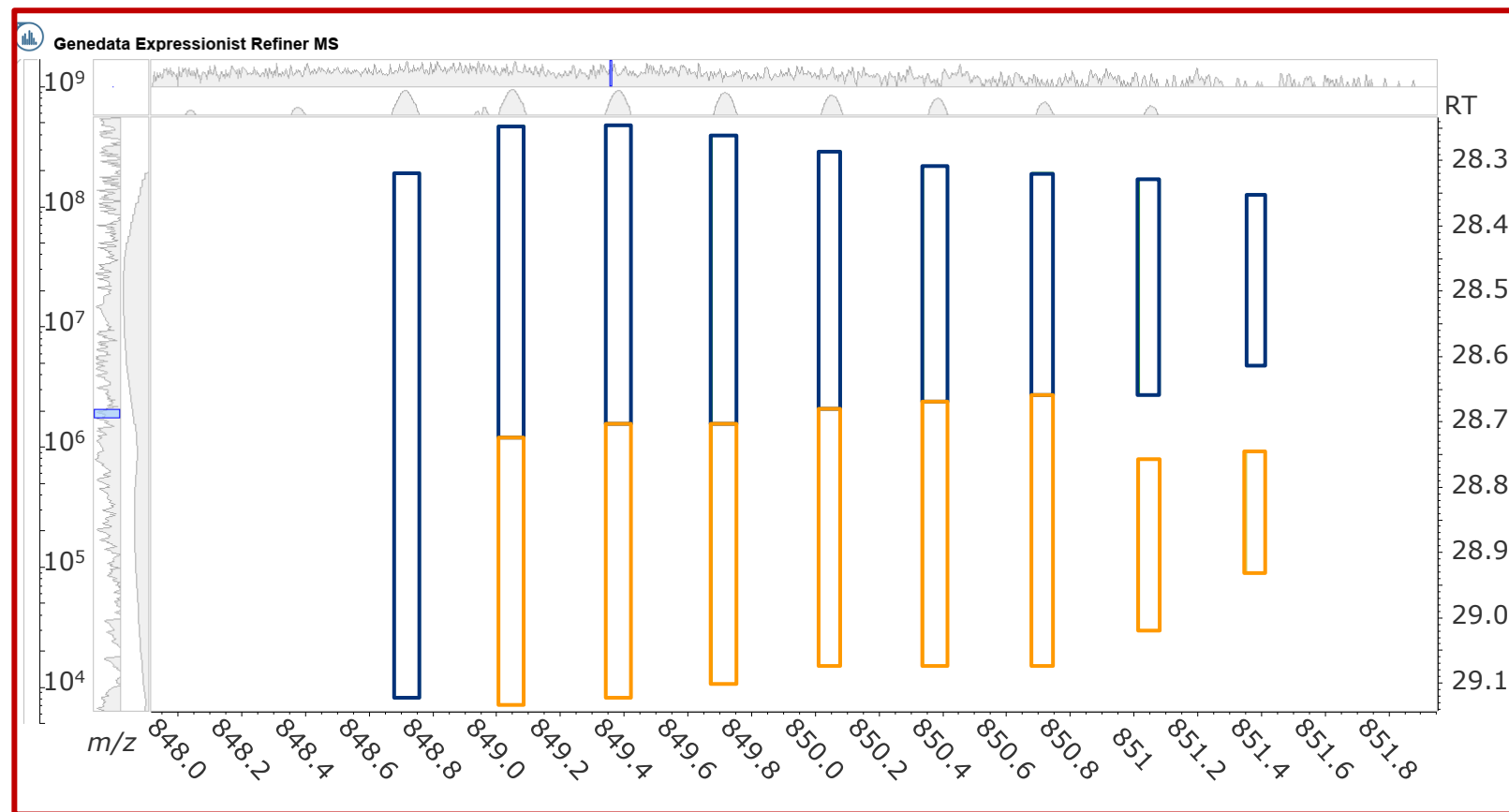
Performed by SME

Manual review of **LC-MS/MS** peptide mapping data from **S/F Study**

Generate **MS1 only** library of **MS2 verified** peptides

Export **Peak Mask**:
RT and m/z coordinates of each peptide in the MS1 only library

Screenshot from Genedata Expressionist Refiner MS



Efficiency: MS1 processing > MS2 processing

Peak Mask Workflow: MS1 Library + Peak Mask

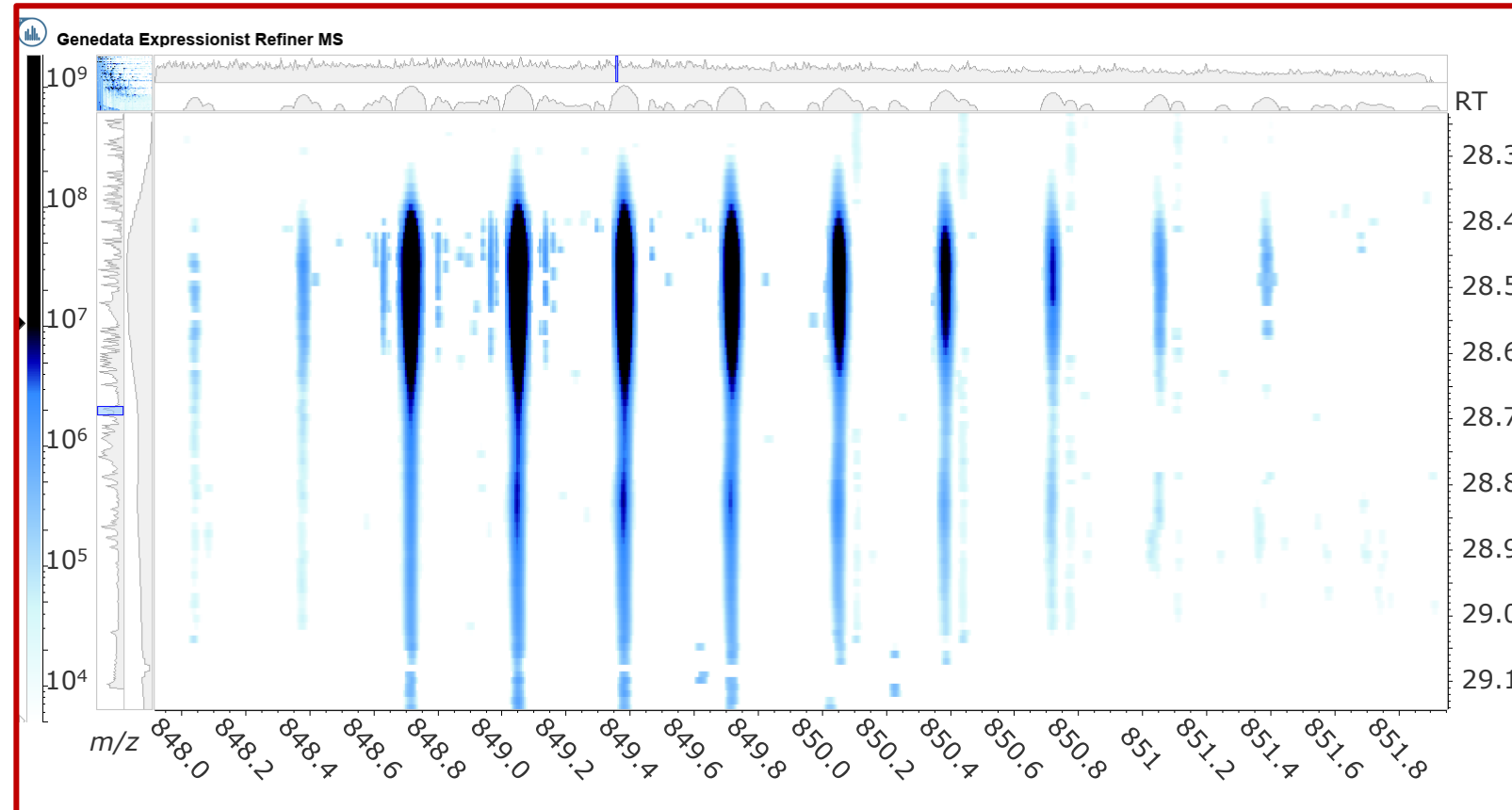
Application of Peak Mask

New data files are **RT aligned** to themselves

Peak Mask is **aligned & overlaid** onto RT Aligned data

Data within **bounding boxes** are kept for further processing & identification

Screenshot from Genedata Expressionist Refiner MS



Efficiency: MS1 processing > MS2 processing

Peak Mask Workflow: MS1 Library + Peak Mask

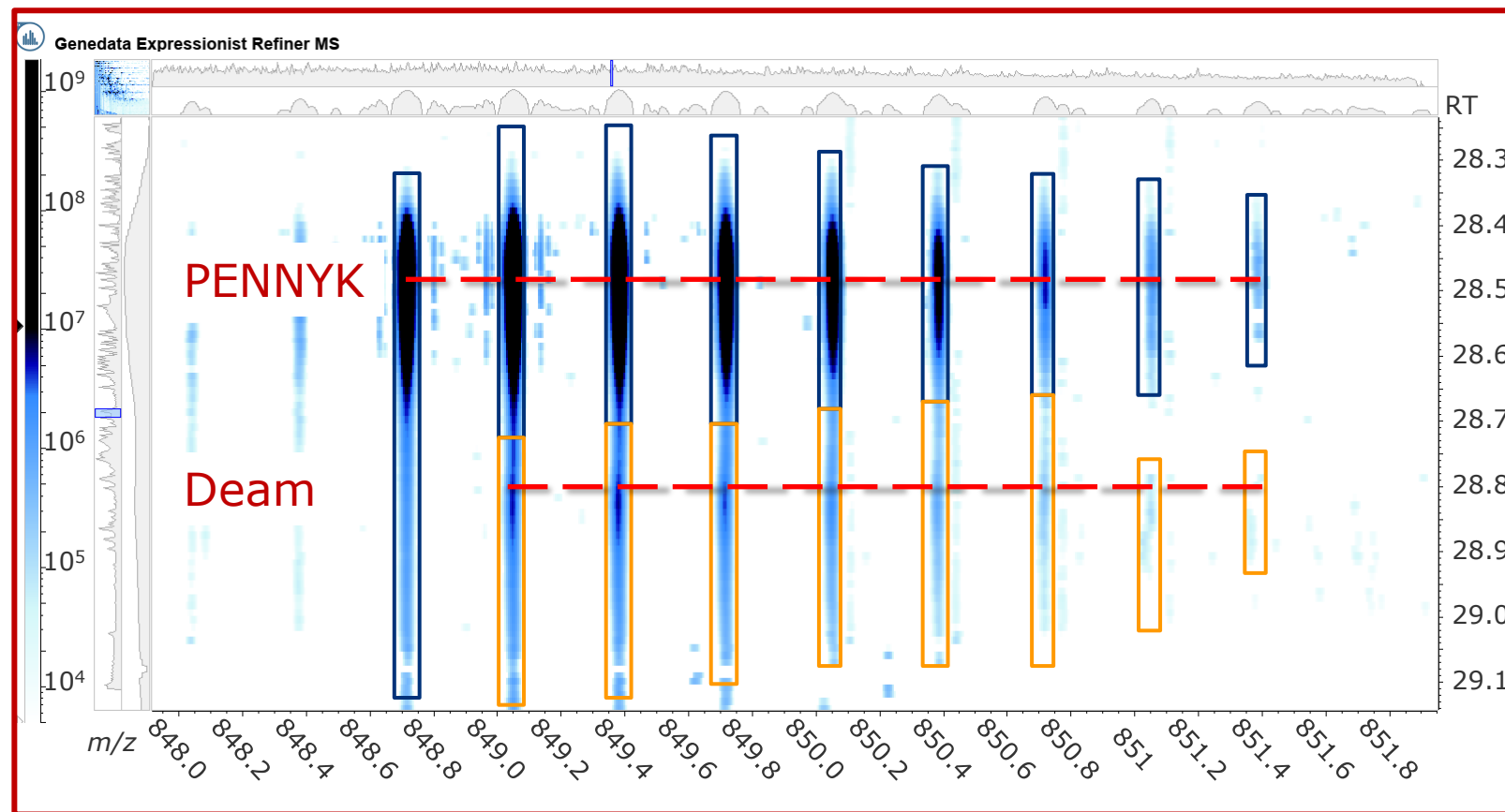
Application of Peak Mask

New data files are **RT aligned** to themselves

Peak Mask is **aligned & overlaid** onto RT Aligned data

Data within **bounding boxes** are kept for further processing & identification

Screenshot from Genedata Expressionist Refiner MS



Efficiency: MS1 processing > MS2 processing

XIC Workflow: MS1 XIC Library

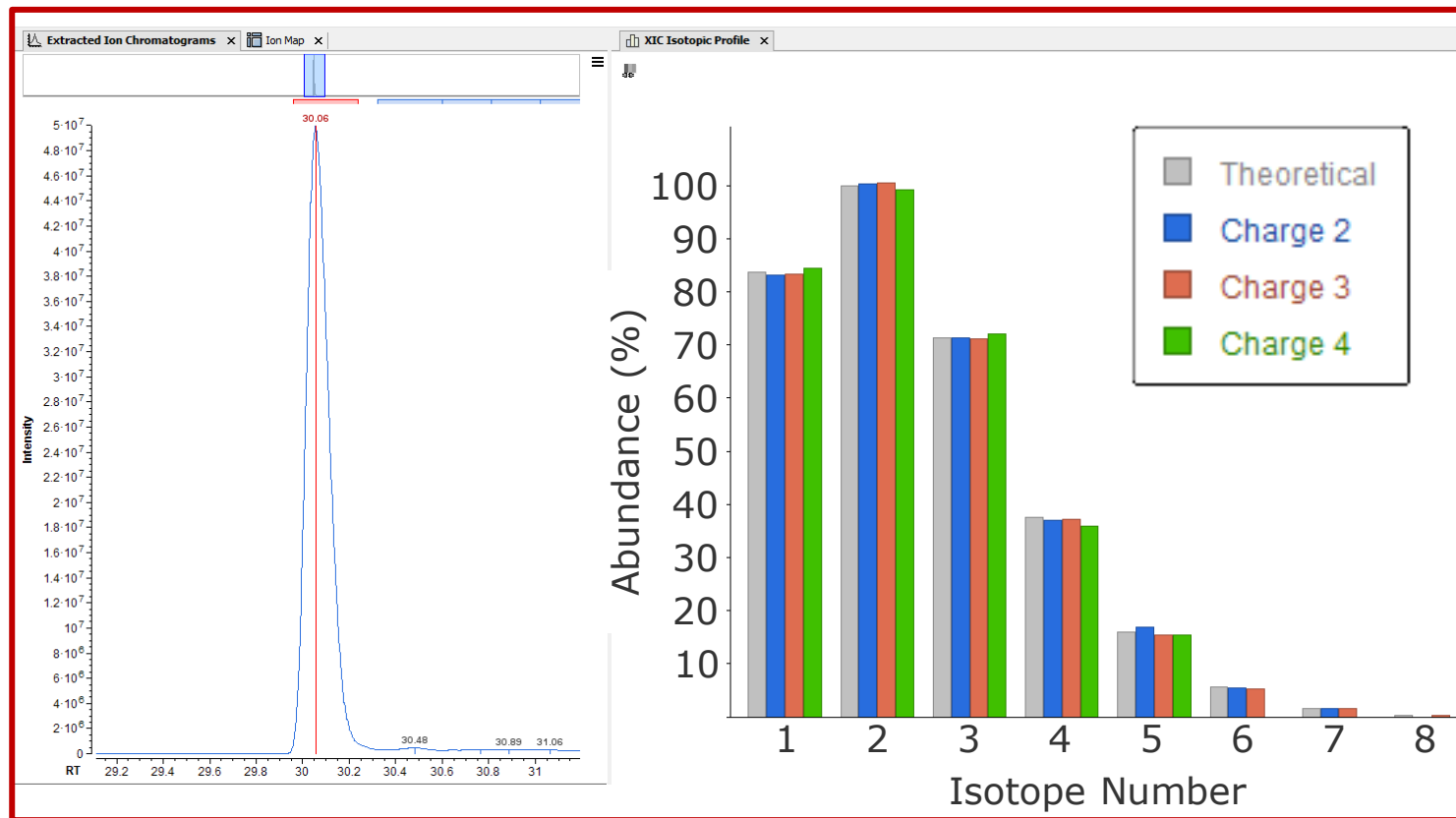
Performed by SME

Export **XIC Library**:
Sequence, RT
+
Specific **charge-states**, and
isotopes to monitor

Application of XIC Library

Data that fit **theoretical isotopic pattern** are kept for further processing & identification

Screenshot from Genedata Expressionist Refiner MS



Efficiency: MS1 processing > MS2 processing

Fully Customizable Template for Automated Analysis & Reporting

日本語

Genedata solutions in silico

Products & Services Knowledge News & Events Careers Company Why Genedata

Contact Us

Digitalizing Biopharma R&D

The Genedata Biopharma Platform is the #1 enterprise software system for achieving operational excellence in biopharma R&D.

Discover Our Platform

25 Years Digitalizing Biopharma 1997-2022

Our Customers

Merck Johnson & Johnson Alexion Bayer Octapharma Evonik Industries Janssen Apteva Therapeutics

Custom plug-ins

Built for:

Peak Mask Workflow
XIC Monitoring Workflow

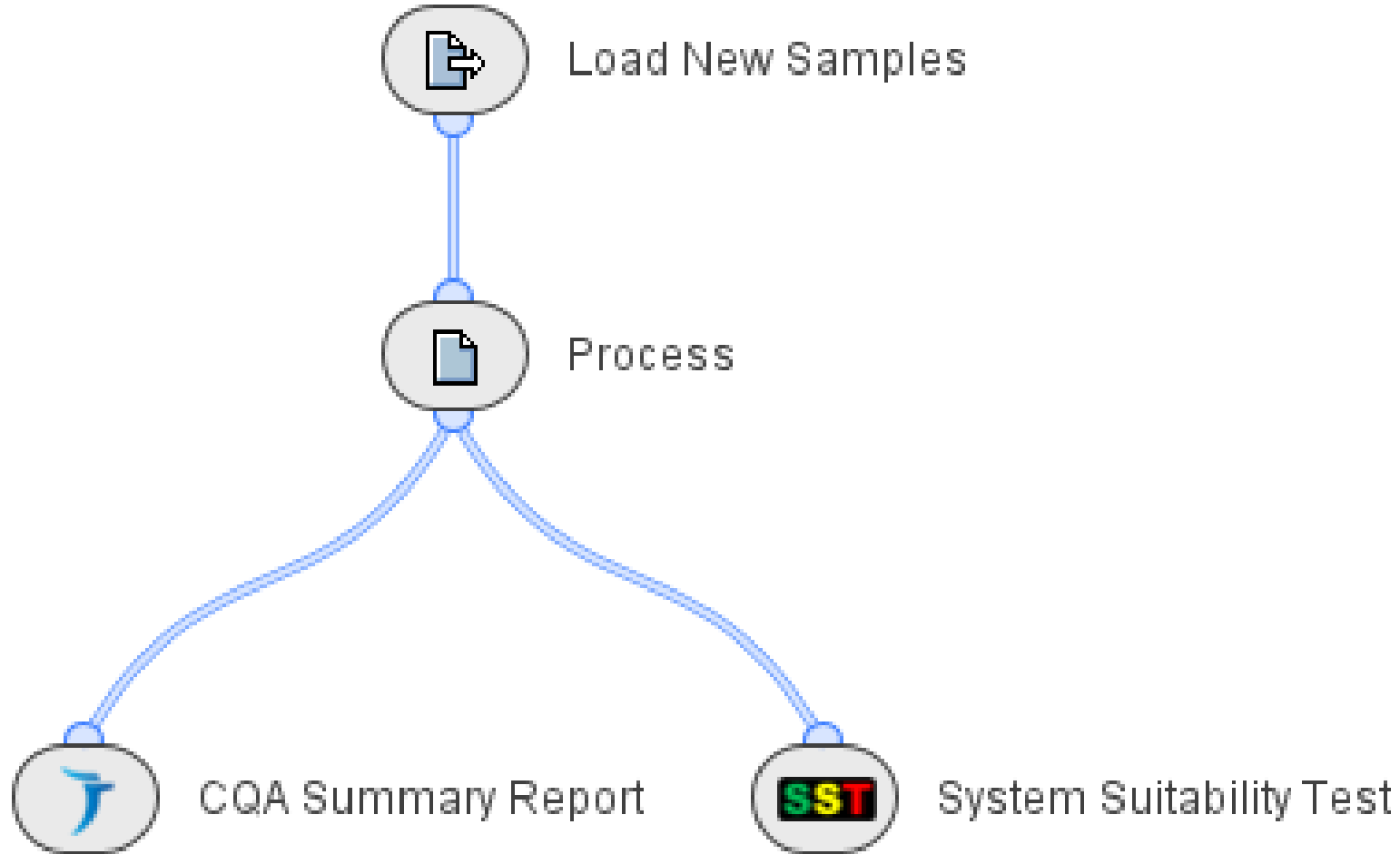
Instructions as .txt files:

Specify Calculations
Report Formatting

Works with:

LC-MS data
LC-MS/MS data*

Automated Reporting: Custom Genedata Plug-ins

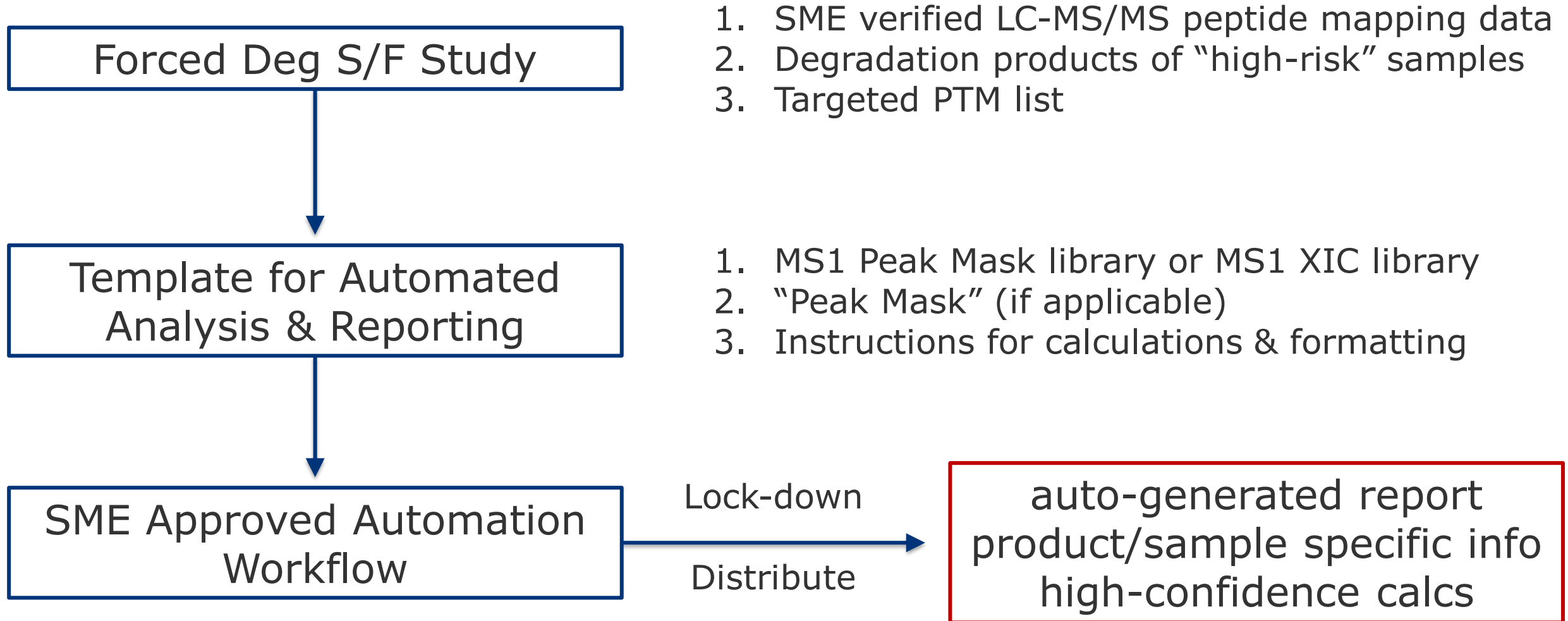


Instructions:

Specify Calculations
Report Formatting

.txt files are used as input
settings for driving
customized specifications

Creating an Automated MS1 Workflow for PTM Monitoring



SME Approved, Locked-down Automation Workflow

Q Search Workflows

Lock-down Most Settings

- MS1 Library (**what**)
- Peak Mask (**where**)
- PTM Calcs (**how**)
- Report Format

Shared

Inbox

Approved

CQA Monitoring Workflow

Demo Workflow (13July21)

Archived

Demo

CQA Monitoring Workflow

Saved by Michael Pettit at Feb 23, 2022, 1:18:48 PM

Workflow approved by Michael Pettit at Feb 23, 2022, 1:17:48 PM with the reason: CQA Workflow Automation

```
graph TD; A[Load New Samples] --> B[Process]; B --> C[CQA Summary Report]; B --> D[System Suitability Test];
```

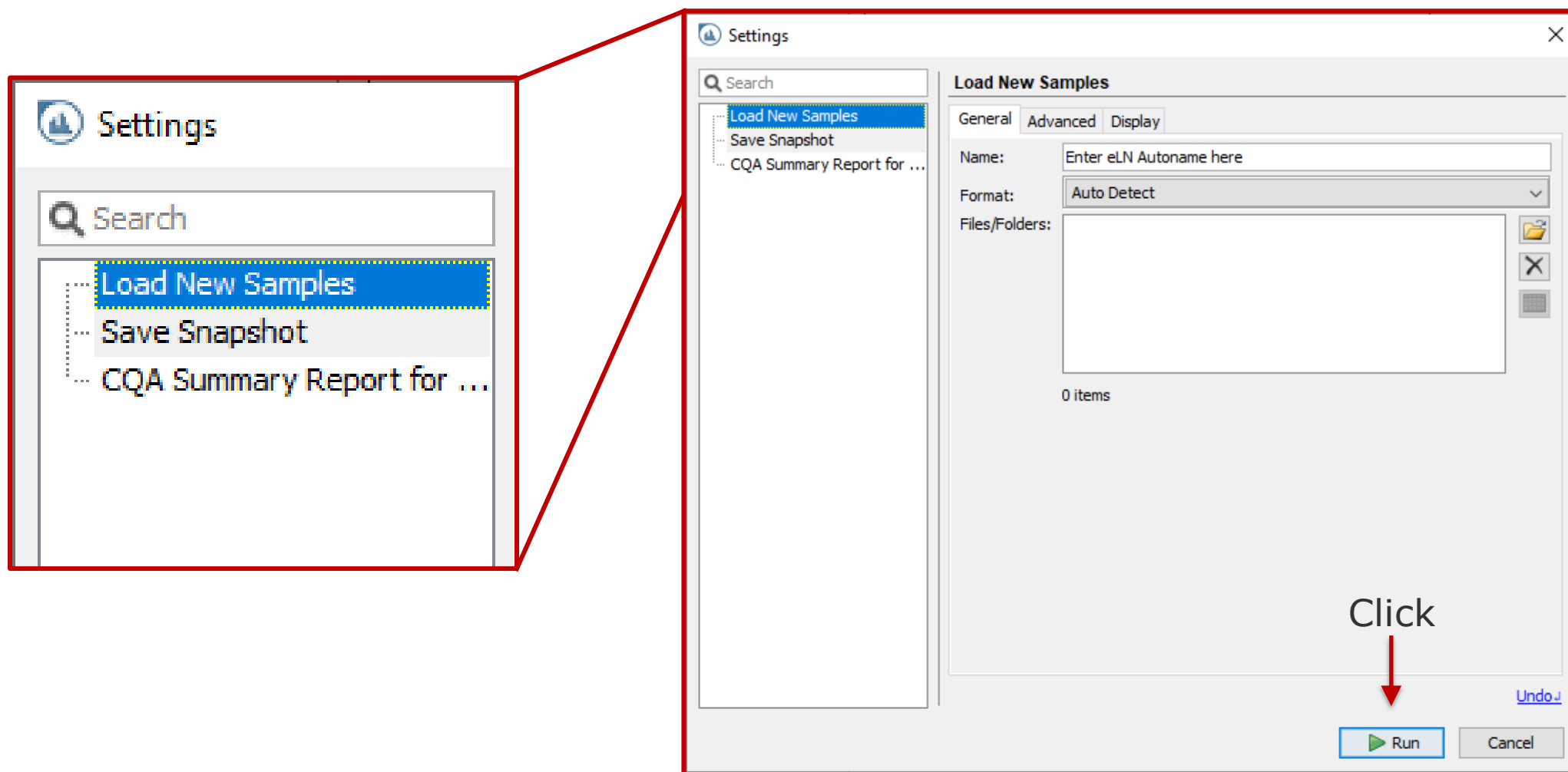
Load New Samples

Process

CQA Summary Report

System Suitability Test

SME Approved, Locked-down Automation Workflow



Auto-generated, Auto-formatted, High-confidence

CQA Summary Report

Summary

Here is the description for the table.

SME approved
structured-data compliant
100% customizable report

Modification	Reference RM1 N/A [%]	Photo 2xICH N/A [%]	Heat 3 Month N/A [%]	High pH 19 Day N/A [%]	Peracetic Acid 1000 uM N/A [%]	AAPH 0.4% N/A [%]	Reference RM2 N/A [%]
Antibody 1 N-term Q Gln->pyro-Glu	81.1	81.7	97.2	100.0	79.7	81.6	82.1
Antibody 1 N-term Q Gln->pyro-Glu	99.3	99.3	99.9	100.0	99.2	99.3	99.3
Antibody 1 M34 Oxidation	0.3	0.3	0.3	0.3	0.4	0.4	0.3
Antibody 1 M103 Oxidation	1.7	25.3	2.8	5.1	25.6	3.7	1.8
Antibody 1 M108 Oxidation	2.2	20.7	2.6	6.0	64.1	3.1	1.8
Antibody 1 W32 Oxidation	0.7	40.1	4.4	1.2	0.9	78.4	0.7
Antibody 1 N55/N59 Deamidation	4.1	3.9	9.9	57.9	4.2	4.5	4.1
Antibody 1 Isomerization	0.5	0.8	5.7	0.6	0.5	2.4	0.5
Antibody 1 Isomerization	0.9	1.1	15.9	3.6	0.8	1.0	0.9
Antibody 1 R61 SV [Arg->Lys]	1.0	1.2	1.2	1.3	1.1	1.1	1.1
Antibody 1 N392/N397 N386/N391 Deamidation	4.5	4.1	7.1	50.8	4.2	4.2	4.7

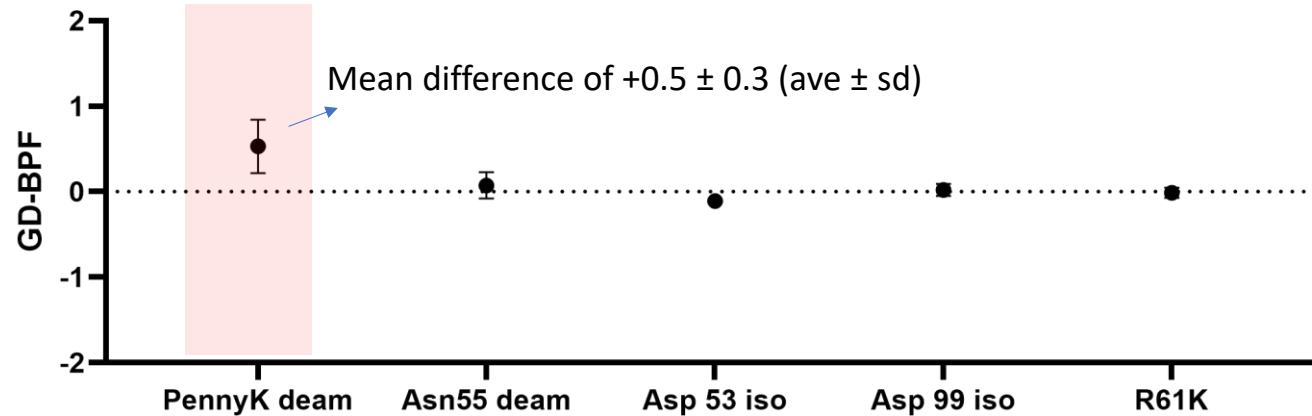
Testing Automated Peak Mask and XIC Workflows against Historical Datasets

LC-MS/MS Peptide Mapping Method Validation:

2 Analysts, 2 Days/Instruments, 6 Samples
(N = 24)

Non-Targeted LC-MS/MS Peptide Mapping (Manual Analysis)

Mean difference (Ave +/- 1SD) N=24 files

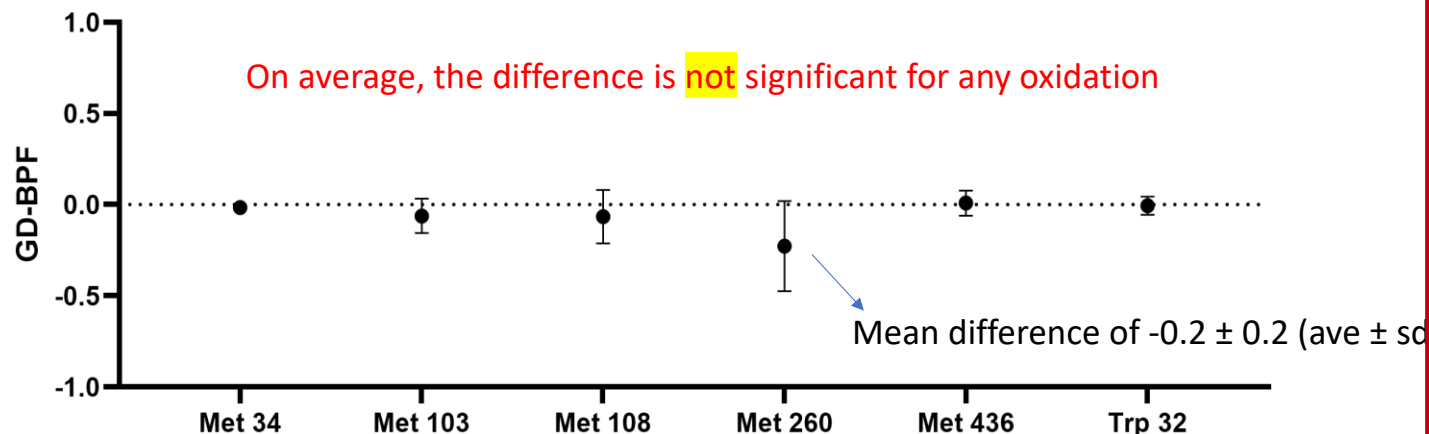


BioPharma Finder & Genedata

1 significant difference (N=24):

PENNYK Deamidation

Mean difference (Ave +/- 1SD) N=24 files



Comparison of Non-Targeted Results vs Automation Results

19.5 GB
LC-MS/MS Data

Manual Method Validation	Automated Peak Mask Workflow	Automated XIC Workflow
4 Datasets Each Processed Separately	4 Datasets Each Processed Separately	4 Data Sets Processed Together
BioPharma Finder	Genedata Refiner MS	Genedata Refiner MS
Used LC-MS/MS Data	Used LC-MS Data	Used LC-MS Data
Manual Analysis & Reporting	Automated Analysis & Reporting	Automated Analysis & Reporting
~ 1 Month	< 2 Hours	< 1 Hour

Performance of Automated Analysis & Reporting

Modification Type	Modification Site	Manual vs Automated Analysis & Reporting (Avg. $\pm$ StDev)		
		Manual (~1 Mo)	PM (<2 Hr)	XIC (<1 Hr)
Deamidation	Ab1 HC Asn 392, Asn 397	2.5 $\pm$ 0.2	3.2 $\pm$ 0.3	2.9 $\pm$ 0.2
	Ab2 HC Asn 55*, Asn 59*	3.5 $\pm$ 0.4	9.1 $\pm$ 1.5	3.2 $\pm$ 0.1
Isomerization	Ab1 HC Asp 53, Asp 54*	0.5 $\pm$ 0.0	0.4 $\pm$ 0.0	0.5 $\pm$ 0.1
	Ab1 HC Asp 99*	1.2 $\pm$ 0.3	1.4 $\pm$ 0.2	1.3 $\pm$ 0.3

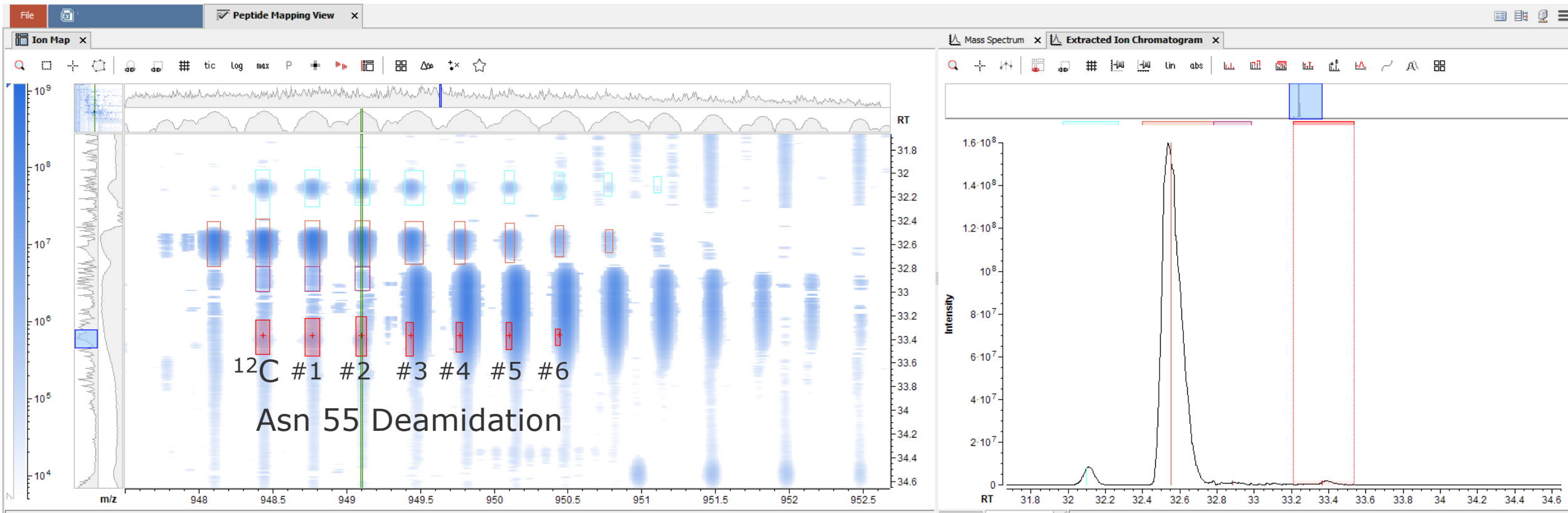
* Site contained within the CDR

Data from LC-MS/MS PepMap Method Validation:
2 Analysts, 2 Days/Instruments, 6 Samples (N = 24)

Isotopic Interference May Impact Quantitation Accuracy

Raw Data Aligned to Peak Mask

XIC of Isotope #2

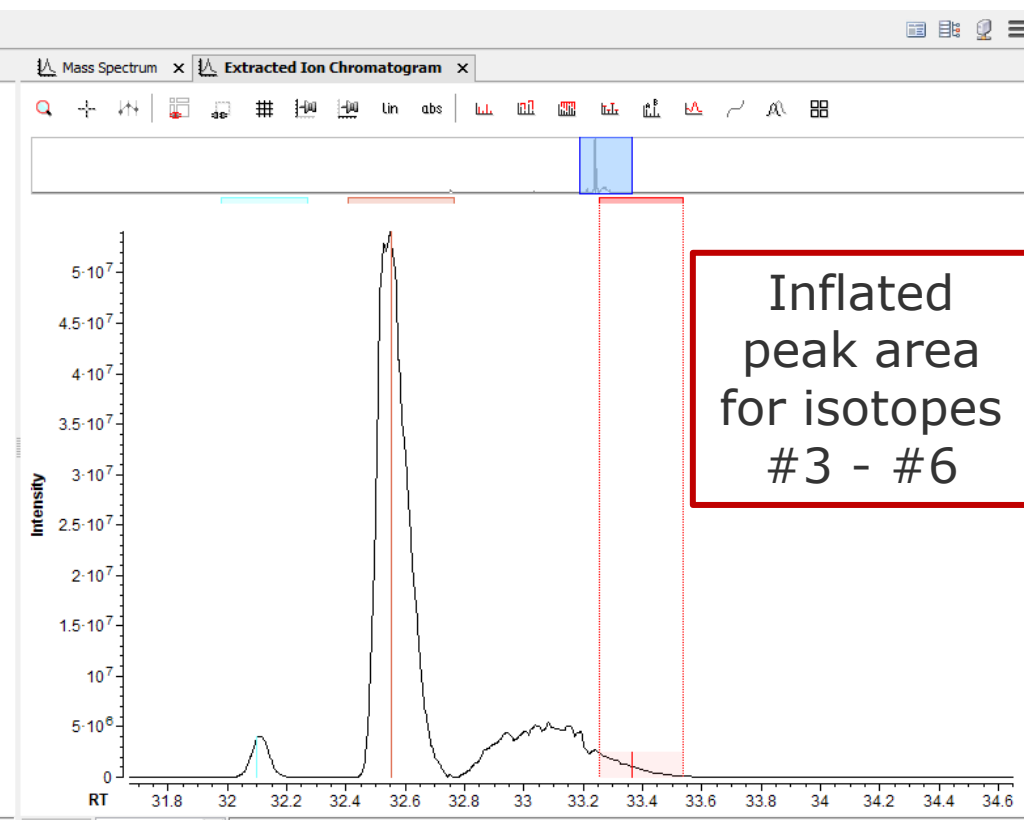
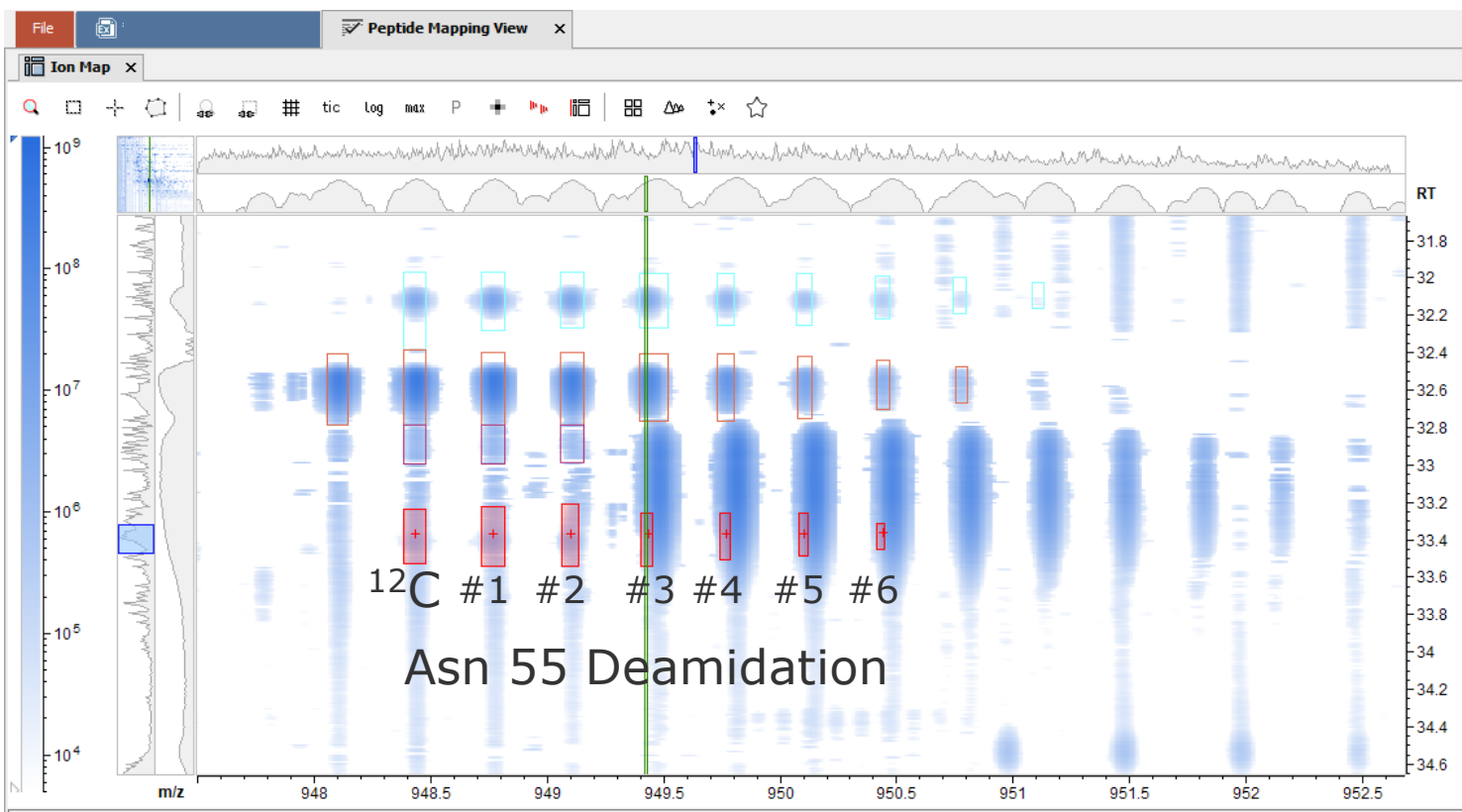


Deamidation at 33.4 mins

Isotopic Interference May Impact Quantitation Accuracy

Raw Data Aligned to Peak Mask

XIC of Isotope #3



PM Workflow = 9.1%
XIC Workflow = 3.2%

(Manual = 3.5%)

Performance of Automated Analysis & Reporting

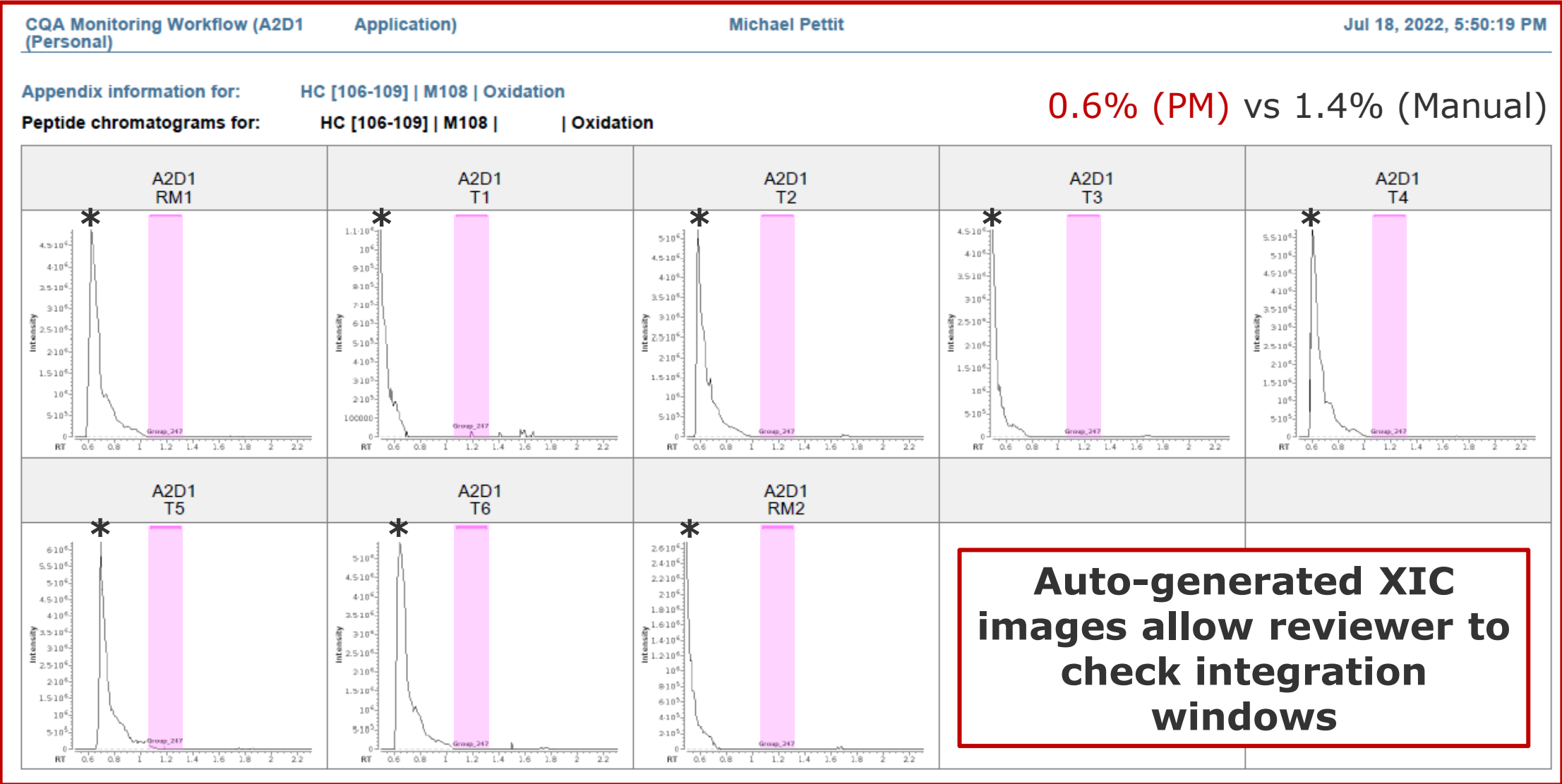
Modification Type	Modification Site	Manual vs Automated Analysis & Reporting (Avg. ± StDev)		
		Manual (~1 Mo)	PM (<2 Hr)	XIC (<1 Hr)
Oxidation	Ab1 HC Met 34*	0.2 ± 0.1	0.1 ± 0.0	0.1 ± 0.1
	Ab1 HC Met 103*	1.3 ± 0.1	1.5 ± 0.1	0.9 ± 0.2
	Ab1 HC Met 108*	1.4 ± 0.2	0.6 ± 0.7	1.5 ± 0.2
	Ab1 HC Met 260	1.8 ± 0.2	1.8 ± 0.1	1.3 ± 0.2
	Ab1 HC Met 436	0.6 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
	Ab2 HC Trp32*/35	1.0 ± 0.1	0.8 ± 0.0	0.7 ± 0.1

* Site contained within the CDR

Data from LC-MS/MS PepMap Method Validation:
2 Analysts, 2 Days/Instruments, 6 Samples (N = 24)

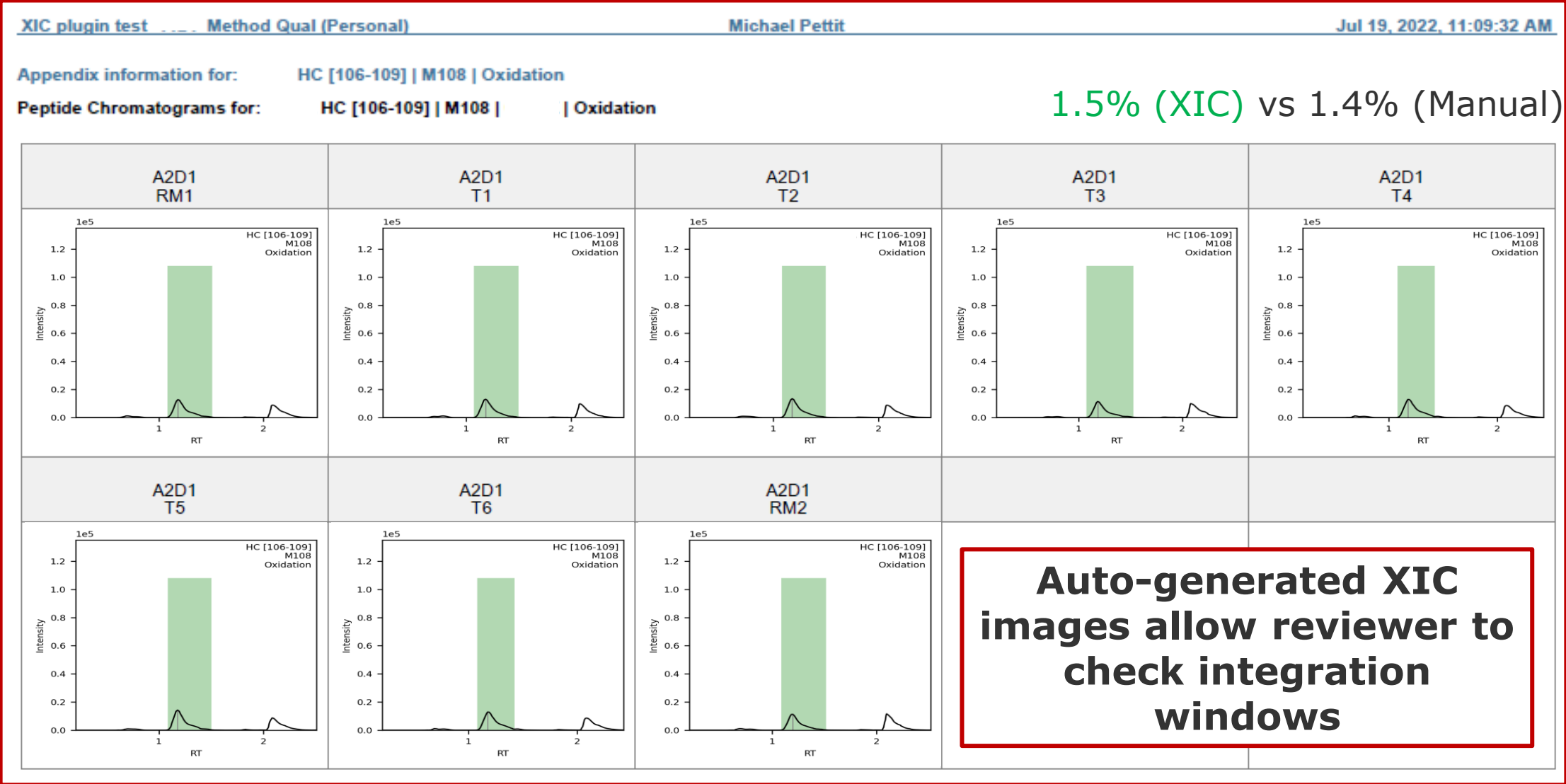
Non-systematic RT Shifts Interfered w/ Peak Mask Workflow

Performance of CQA Workflow $\propto$ Alignment of Raw Data to Peak Mask

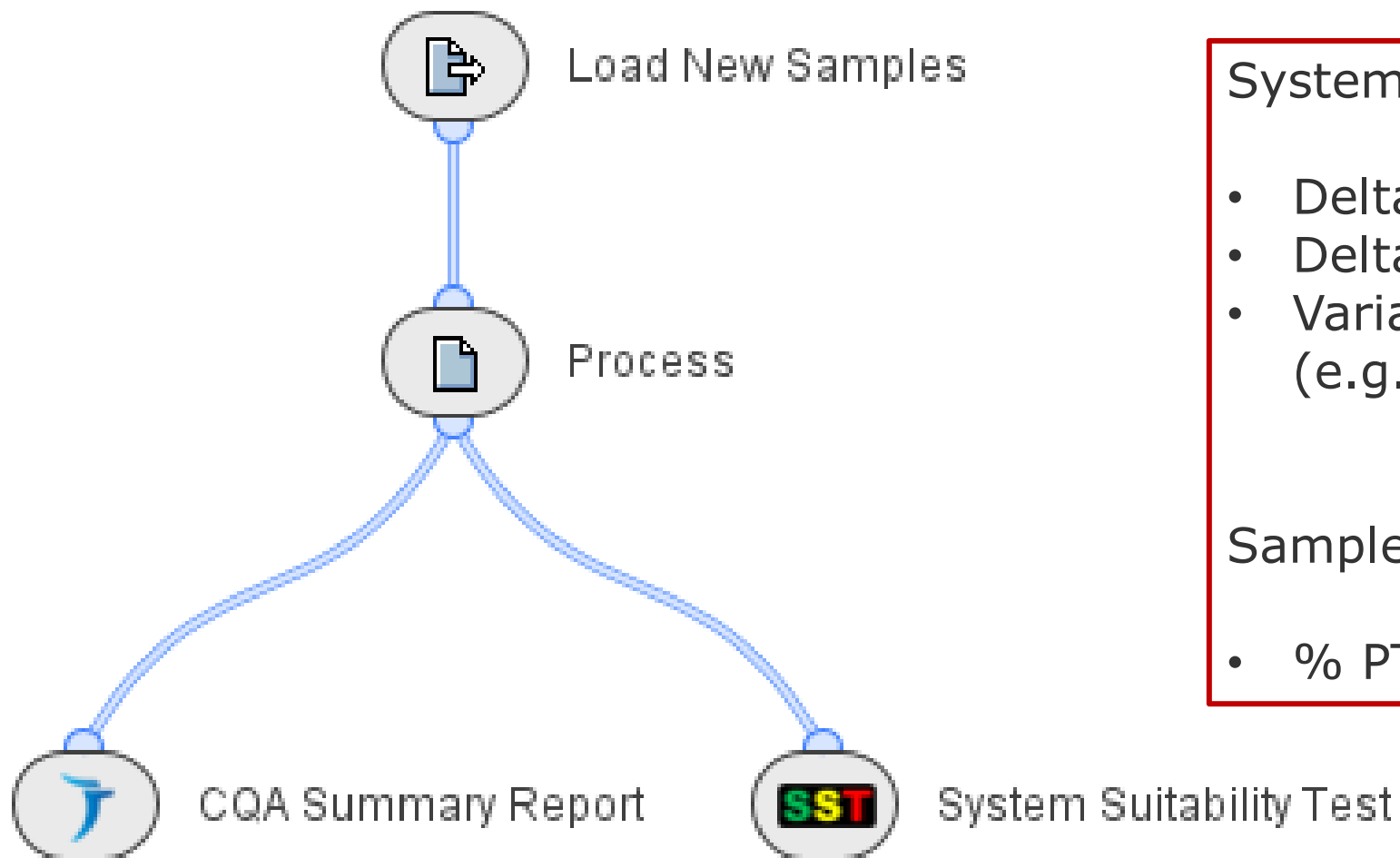


XIC Workflow Performed Well Despite Non-systematic Shifts

Performance of XIC Workflow $\propto$ Isotope Matching



System Suitability Report Improves Confidence in Automation



System Suitability:

- Delta Mass (e.g., Δ 5 ppm error)
- Delta RT (e.g., Δ 0.3 mins)
- Variation of Absolute MS Intensity (e.g., 1.0% RSD)

Sample Suitability:

- % PTM vs Historical Value

System Suitability Report Improves Confidence in Automation

System Suitability Test

Summary

Percent Modification (Max Deviation: $\pm 0.5\%$)

PTM "A"

Data Filename	% PTM Experiment	Δ Historical Value (1.8%)	Outcome
Data File_First Injection	2.1	0.3	Pass
Data File_Last Injection	2.7	0.9	Fail

Conclusion: Automation Enhanced Workflow Efficiency



Significant amount of front-end development by SME

1. FD S/F Study
2. PM or XIC Workflow
3. Customized Plug-ins

4. SME Locked-down Template
5. XIC Images for Truncation
6. SST Report

Analysis & Reporting was automated

Manual Review & Approval was reduced

Bright Future for End-to-End Automation



Acknowledgements

Janssen R&D, Analytical Development

Mehul Patel
Ping Hu
Mike Tang
Mike Lewis

Joe Mulholland
Jingwen Ding
Adam Evans
Weihan Wang
Jihyeon Lim

Genedata Expressionist

Nick Degraan-Weber

Albert van Wyk

