Characterization and Quantitation of Antibody Drug Conjugates

Matthew Blatnik, Ph.D. and Chris Turck, Ph.D.

Pharmaceutical Interest Group Workshop, ASMS Vancouver

Monday, 21 May 2012, 5:45-7:25, Room 109

Number of Attendees: ≥125 people

Workshop Format

Chris and I decided to run the workshop differently this year. We did not like the original format (which we used in 2011) that included three short panelist lead presentations followed by open discussion (we were coached on using this format). Our panelists in 2011 talked longer than their allocated 10 mins, interest decreased and we agreed that most folks were tired of listening to talks by the end of the day.

We altered our 2012 survey to include open ended questions along with solicitation for panelists willing to share and engage an audience. From the names provided, ~ 50 people were interested in participating. We cross referenced these individuals using Medline and LinkedIn to find experts. We asked Ola Saad (Genentech) to provide a 25 min presentation overview of the topic and asked the remaining experts to provide a statement of intent by email for participating as discussion panelists. Ryan Preston (Pfizer CovX), Jennifer Nemeth-Seay (J & J) and Jinzhi Chen (Takeda) provided very nice responses and joined Ola after her presentation as panelists. We asked the panelists to discuss the topic between them to keep dialog going if the audience was silent.

Workshop Discussion

Ola gave a 30 min overview of the topic which included: characteristics of ADC biologics, conjugation sites/load and fit-for-purpose assay strategies including ELISA and LC-MS as complimentary tools. The panel/audience led discussion included: peptide mapping of ADC's, selecting conjugation sites (working around IP/patents), differentiating conjugation sites and drug antibody ratio (DAR) in vivo/ex vivo, linker stability/toxicity and preclinical species radio labeling experiments. One of the biggest issues in the ADC space is the translation between ELISA and LC-MS (with DAR and free payload) and PK-PD modeling.

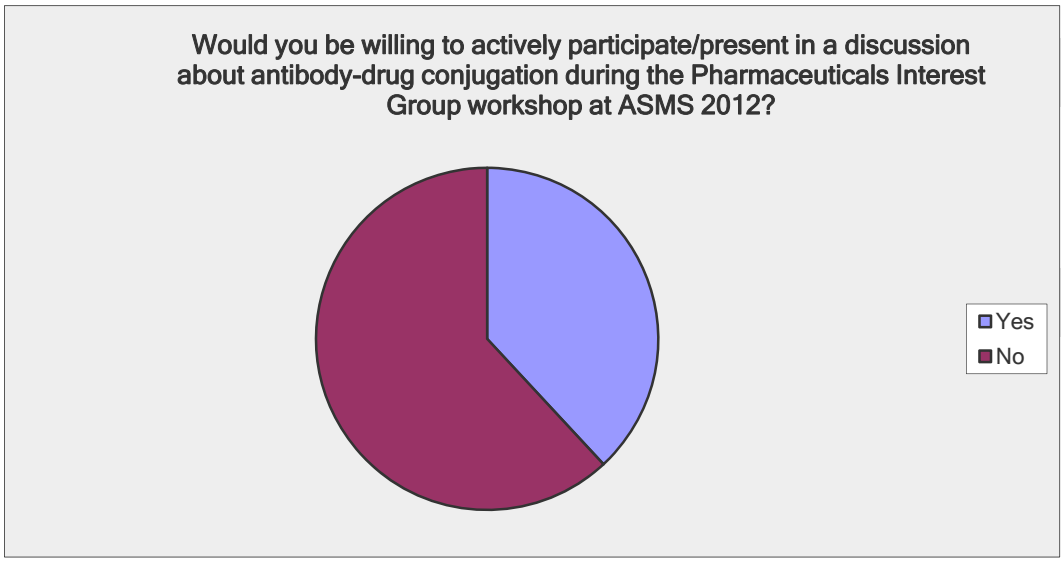
The discussion following Ola's overview was very constructive. There was a lot of participation and we ran over 20 mins.

Succession Plan

Using more of an Egalitarian approach to the workshop, Chris and I want to submit a call for workshop leaders to all the members of the pharma group via email (including the ~ 30 new members we picked up this year) and ask them to write a statement of intent (no more than 100 words). We have made positive impacts with this group over the last two years and want to make certain it continues to thrive by choosing people interested in putting some time. Therefore, we want to identify our successors but stay on as workshop leads for 2013 to properly coach and mentor the new leaders. We will introduce them as our successors at next year's workshop (if we are allowed to have one).

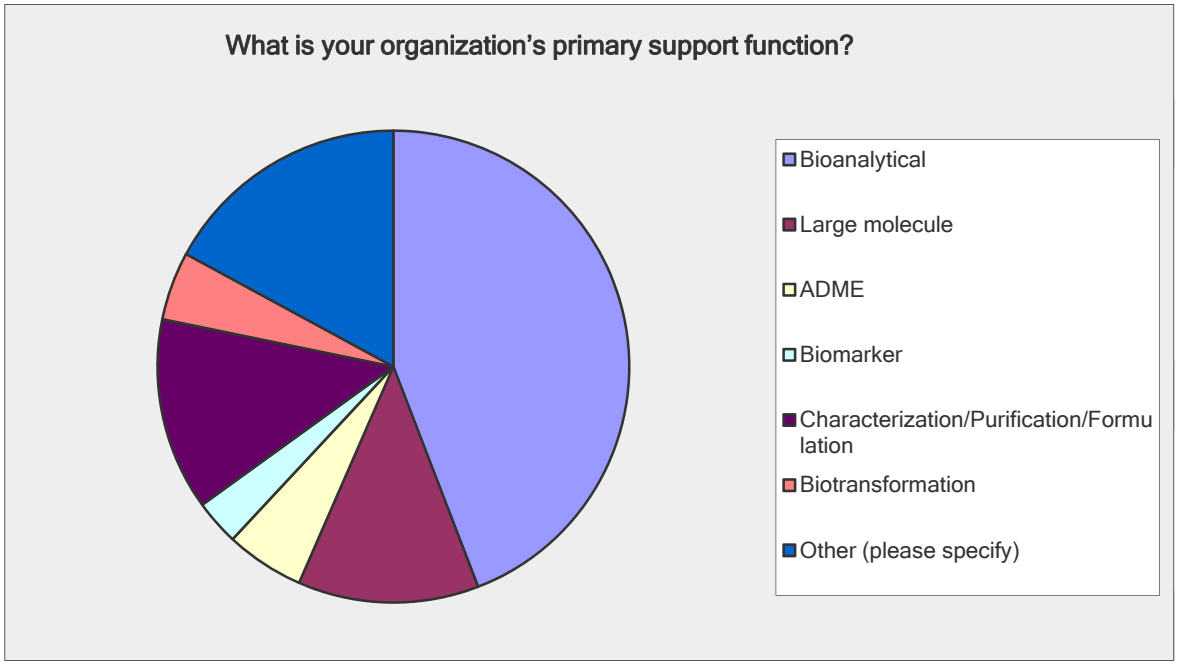
ASMS 2012 Pharma IG

Would you be willing to actively participate/present in a discussion about antibody-drug conjugation during the Pharmaceuticals Interest Group workshop at ASMS 2012?		
Answer Options	**Response Percent**	**Response Count**
Yes	38.1%	56
No	61.9%	91
	answered question	**147**
	skipped question	**1**



ASMS 2012 Pharma IG

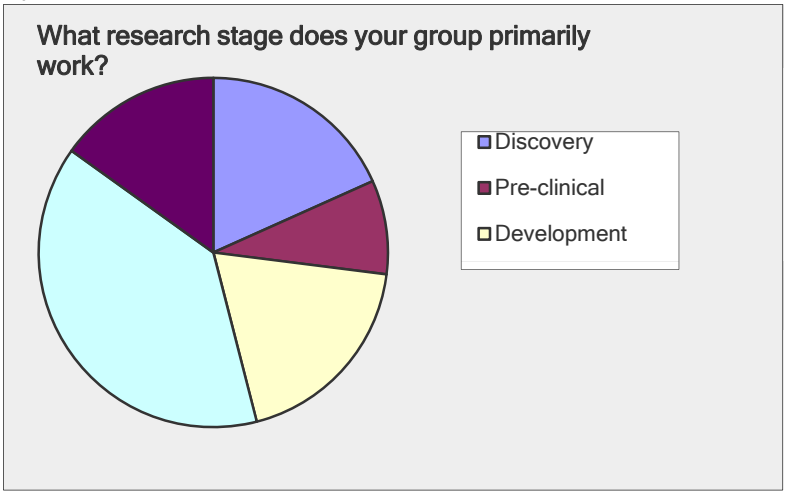
What is your organization's primary support function?		
Answer Options	**Response Percent**	**Response Count**
Bioanalytical	44.2%	57
Large molecule	12.4%	16
ADME	5.4%	7
Biomarker	3.1%	4
Characterization/Purification/Formulation	13.2%	17
Biotransformation	4.7%	6
Other (please specify)	17.1%	22
	answered question	129
	skipped question	19



ASMS 2012 Pharma IG

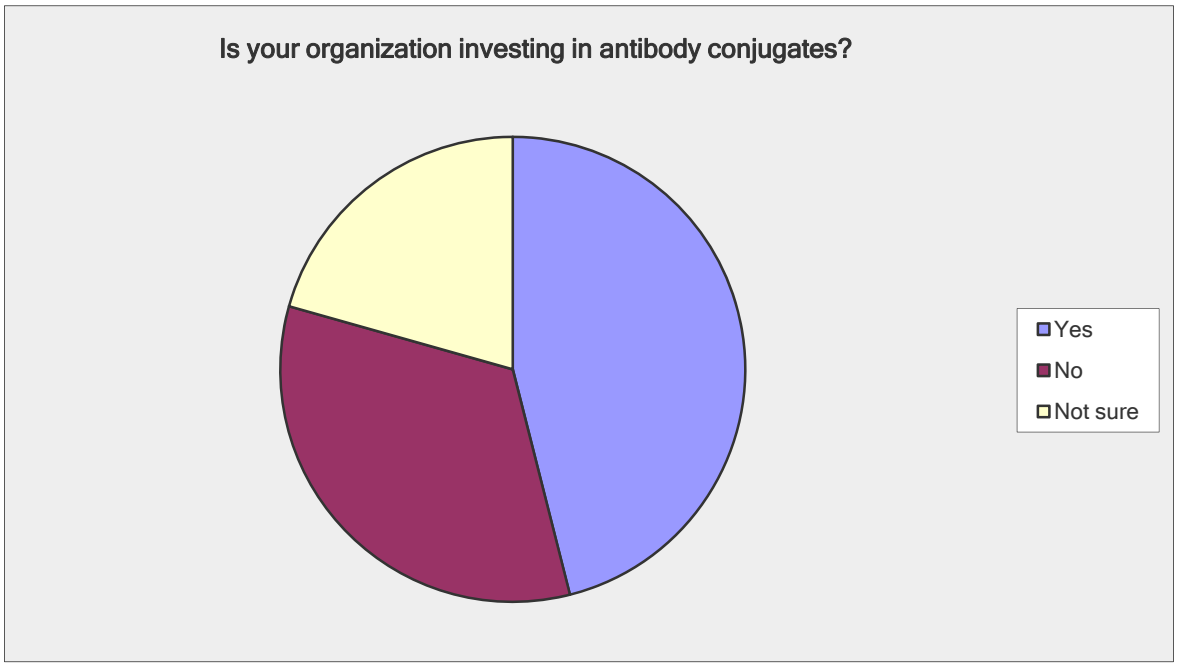
What research stage does your group primarily work?		
Answer Options	**Response Percent**	**Response Count**
Discovery	18.3%	23
Pre-clinical	8.7%	11
Development	19.0%	24
A combination of above	38.9%	49
Other (please specify)	15.1%	19
	answered question	126
	skipped question	22

Other (please specify)
Support of all
Clinical trials
clinical
CRO
basic research
Law
LC/MS method development and optimization
INstrument Vendor
proteomics
glyco conjugates
instrument vendor
We regularly collaborate with customers in all of these areas.
support across the development pipeline
CRO, all stage
method develpoment
Instrument Vendor
Regulatory, Quality, Safety
all stages
authenticity studies



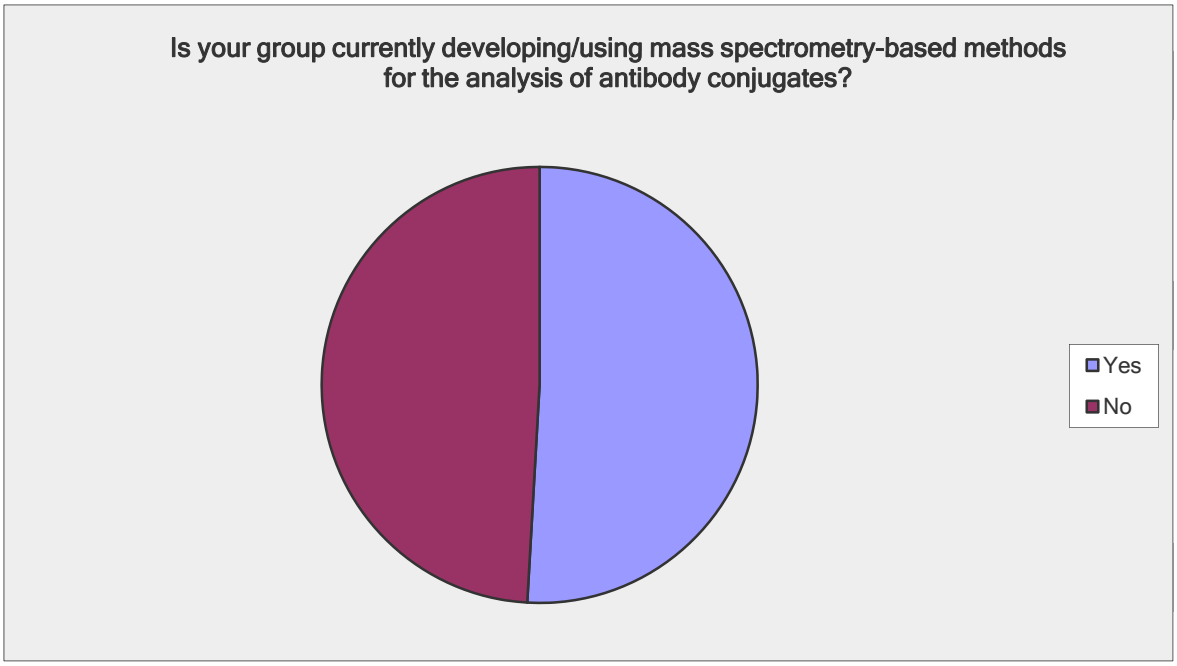
ASMS 2012 Pharma IG

Is your organization investing in antibody conjugates?		
Answer Options	**Response Percent**	**Response Count**
Yes	46.0%	58
No	33.3%	42
Not sure	20.6%	26
	answered question	126
	skipped question	22



ASMS 2012 Pharma IG

Is your group currently developing/using mass spectrometry-based methods for the analysis of antibody conjugates?		
Answer Options	**Response Percent**	**Response Count**
Yes	50.9%	58
No	49.1%	56
	answered question	114
	skipped question	34



ASMS 2012 Pharma IG

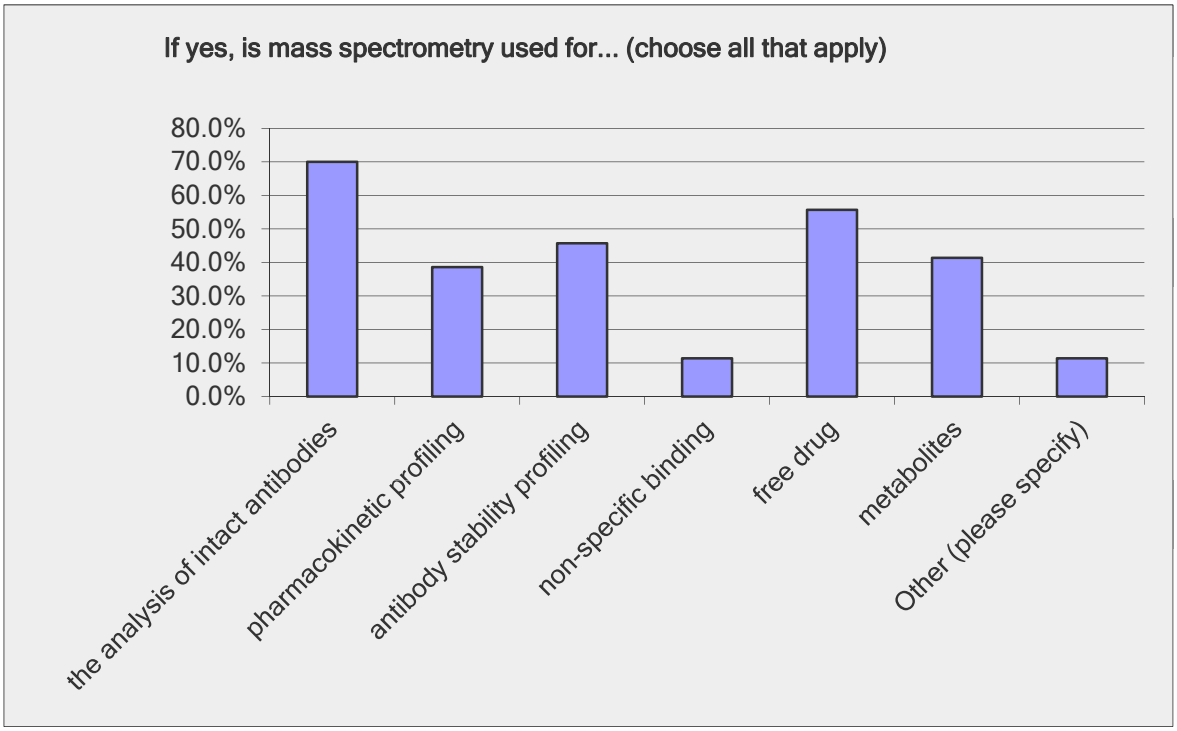
If yes, would you be willing to discuss generic challenges and resolutions during the Pharmaceuticals Interest Group workshop at ASMS 2012?	
Answer Options	**Response Count**
	31
answered question	31
skipped question	117

Response Text
yes...
Yes
sure, discuss but not present
Yes. As an executive director I could provide a high level overview of challenges and opportunities.
Yes. Biosimilars analysis is critical for the industry
yes
yes
I am still a beginner and not proficient in this field.
Yes. As an executive director of an analaytical group, I would discuss this at a more strategic level.
Yes
yes
no
Yes. We would like to discuss our experience and findings about ADCs.
i'm not going to ASMS this year
MS inlet methods - chromatography (SEC, RP, etc.)MS analysis - full conjugate - Drug antibody ratio, site cha
We just started with this type of analysis. The main focus is to identify antibody conjugates generated by biotr
Yes
if I am allowed to do so by our company IP Dept
yes
yes
Depends on signed NDAs. Might not be able to discuss much.
yes
No
no, sorry, have not received clarance on this.
Yes
depends on the company's confidence
I would be interested in listening and learning more about the issues
Yes
yes
No, we do not use them
No

ASMS 2012 Pharma IG

If yes, is mass spectrometry used for... (choose all that apply)		
Answer Options	**Response Percent**	**Response Count**
the analysis of intact antibodies	70.0%	49
pharmacokinetic profiling	38.6%	27
antibody stability profiling	45.7%	32
non-specific binding	11.4%	8
free drug	55.7%	39
metabolites	41.4%	29
Other (please specify)	11.4%	8
	answered question	70
	skipped question	78

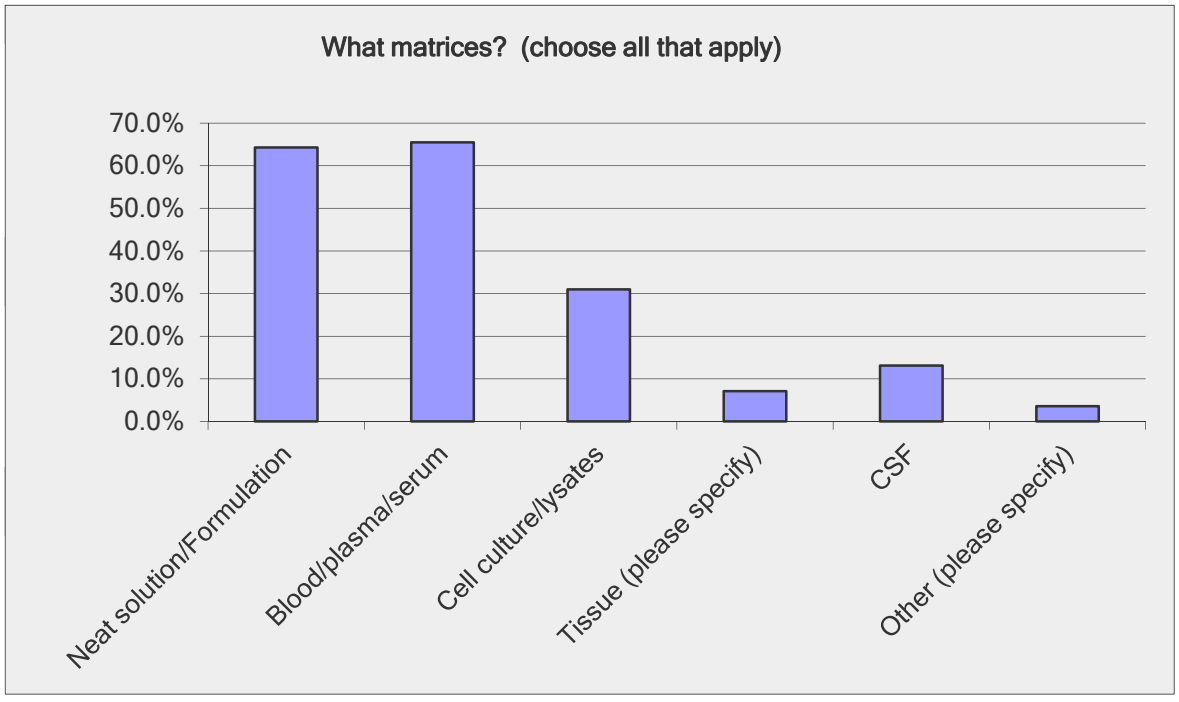
Other (please specify)	Categories
All of the above	
HDX-MS	
glyco conjugates	
proteomics	
small molecule	
Warhead characterization, intact conjugated antibodies, process chemistry support of chemical modifica	
mass spectral analysis of oligo deamination degradants without separation	
conjugation sites	



ASMS 2012 Pharma IG

What matrices? (choose all that apply)		
Answer Options	**Response Percent**	**Response Count**
Neat solution/Formulation	64.3%	54
Blood/plasma/serum	65.5%	55
Cell culture/lysates	31.0%	26
Tissue (please specify)	7.1%	6
CSF	13.1%	11
Other (please specify)	3.6%	3
	answered question	84
	skipped question	64

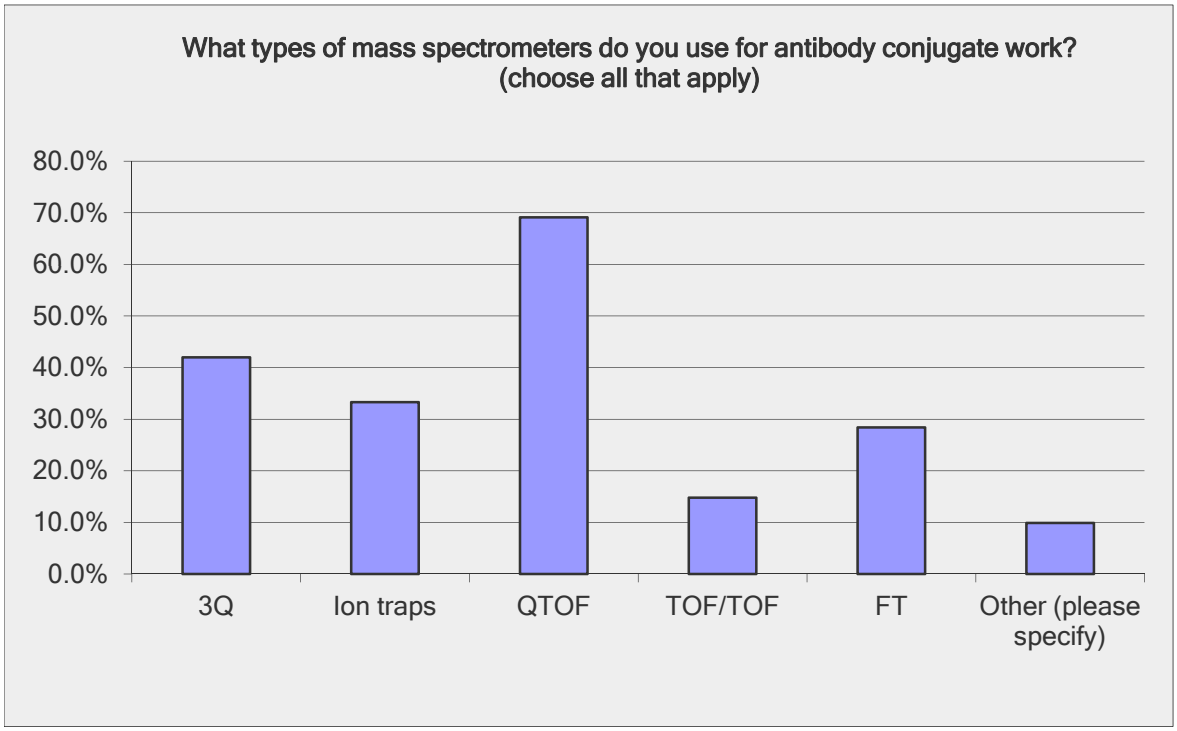
Number	Response Date	Other (please specify)	Categories
1	Mar 1, 2012 5:06 PM	Tissue and urine for signs of adduct formatior	
2	Feb 10, 2012 11:53 AM	microarrays and tissues	
3	Feb 6, 2012 8:44 PM	glutathione stabilty for cystein conjugates	



ASMS 2012 Pharma IG

What types of mass spectrometers do you use for antibody conjugate work? (choose all that apply)		
Answer Options	**Response Percent**	**Response Count**
3Q	42.0%	34
Ion traps	33.3%	27
QTOF	69.1%	56
TOF/TOF	14.8%	12
FT	28.4%	23
Other (please specify)	9.9%	8
	answered question	81
	skipped question	67

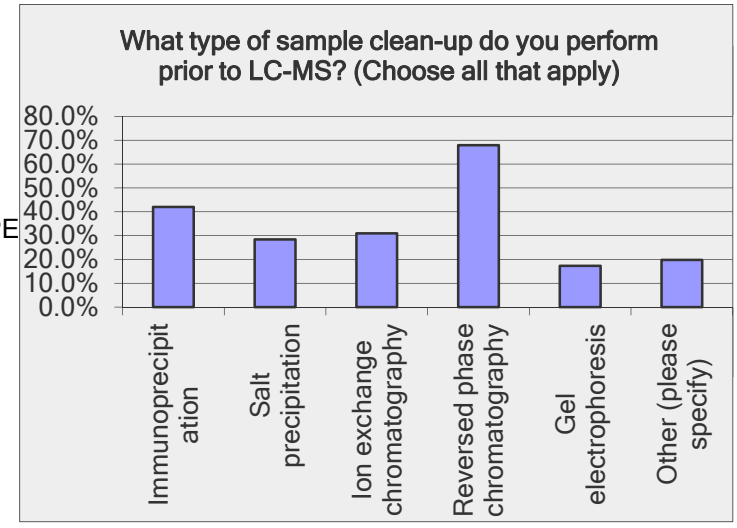
Other (please specify)	Categories
orbi	
Other hybrids	
GEMMA	
Not studying antibody conjugates yet	
tof	
Orbitrap for FC-Conjugates	
ortial traps	
Orbi	



ASMS 2012 Pharma IG

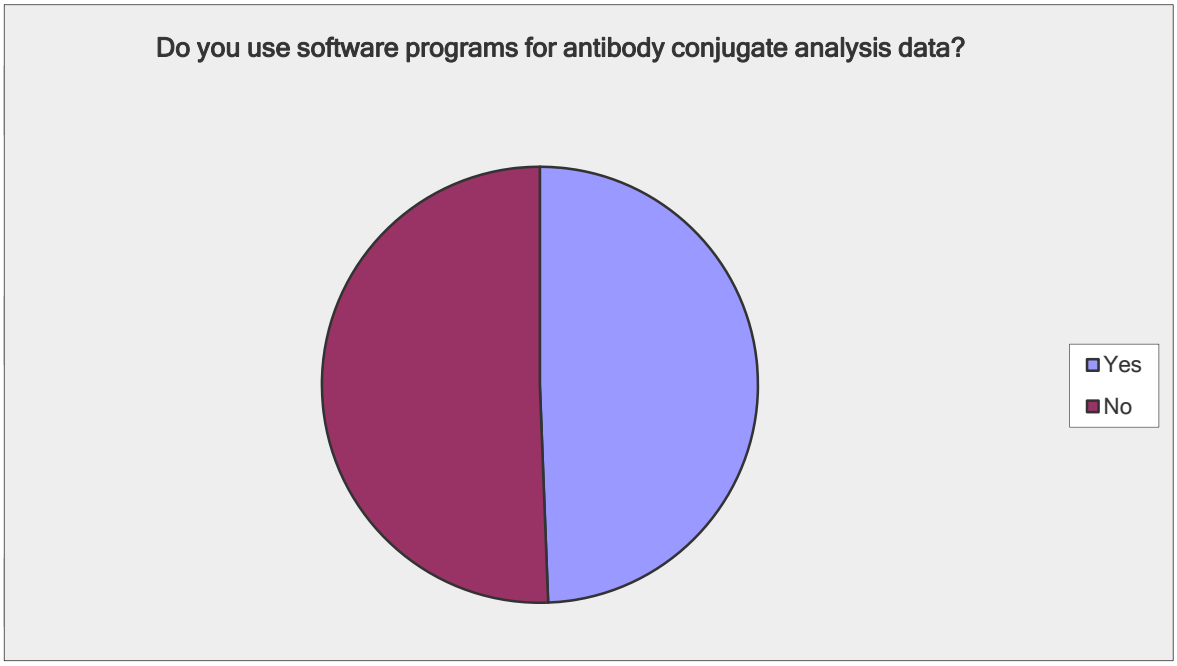
What type of sample clean-up do you perform prior to LC-MS? (Choose all that apply)		
Answer Options	**Response Percent**	**Response Count**
Immunoprecipitation	42.0%	34
Salt precipitation	28.4%	23
Ion exchange chromatography	30.9%	25
Reversed phase chromatography	67.9%	55
Gel electrophoresis	17.3%	14
Other (please specify)	19.8%	16
	answered question	81
	skipped question	67

Other (please specify)
SPE
none
protein precipitation
SPE and Buffer exchange
DBS
SPE of digests
Dialysis
protein precipitation, liq-liq extraction, SPE
dialysis
none
Solid phase microextraction
SEC
Turbulent Flow Chromatography
Surfactant removal
PGC cartridge
DBS



ASMS 2012 Pharma IG

Do you use software programs for antibody conjugate analysis data?		
Answer Options	**Response Percent**	**Response Count**
Yes	49.4%	42
No	50.6%	43
	answered question	85
	skipped question	63



ASMS 2012 Pharma IG

If yes, please state for what application (mass deconvolution, quantitation, etc.)

Answer Options	Response Count
	34
answered question	34
skipped question	114

Response Text
mass deconvolution, quantitation
mass deconvolution, peptide mapping
quantitation
Bruker BioTools, for intact mass deconvolution, and peptide mapping (with modifications specified)
mass deconvolution and peptide ID
Mass deconvolutionPeptide maps analysis
Various
Mass decon
Mass hunterProteome discoverer
mass deconvolution, PTM identification, de novo sequencing, identification of truncations (metabolites) of the
BioAnalyst
MassLynx, Biopharmalynx
Mass deconvolutionquantitation
deconvolution
Deconvolution, characterization
mass deconvolution
Intact MS deconvolutionquantitation
Deconvoltution
biopharmalynx
MaxEnt, Protein Deconvolution (new from thermo)
BioPharmaLynx 1.3 from Waters but also various software available from academia.
Mass deconvolution
a unified software for both spectral deconvolution and relative quantitation
ProMass, ThermoFisher
deconvolution
decon
deconvolution, peptide mass mapping
maxent (through Bruker compas) for deconvolution. Excel for quantitation.
Mass deconvolutionDrug distribution prediction
Masslynx
deconvolution, quantitation
all
mass deconvolution
Quantitation

ASMS 2012 Pharma IG

What challenges would you like this workshop to address about antibody drug conjugates and mass spectrometry?	
Answer Options	**Response Count**
	42
answered question	**42**
skipped question	**106**

Response Text
Quantitative analysis of conjugation levels
accuracy for quantitation; matrix effect; the efficiency of trypsin digestion.
Balance of Qual / Quant and specific approaches for each separately and together
Characterization (peptide mapping).
sample prep and analytical tips on what works well; compatibility of sample prep and analysis re: intact Ab vs
Conversion efficiency to release loaded drugs from mAb's and compensation by stable label IS (released pe
sample cleanup/sensitivity
Regulatory expectations for characterization and quanitification of adduct variants in drug product/drug subs
New and novel techniques to elucidate interactions
characerization and quantitation and extraction from complex biological matrices.
Antibodies with large protein conjugates.
Identification of biotransformation products of antibody drug conjugates in plasma from animals and human.
Regulatory requirements/expectations for characterization, PK
binding sites, affinity, screening,
What needs to be quantified: Free drug? Total Ab? Total drug (bound+ free)? AB-Drug conjugates only?
Define the challenges, describe successful approaches to quantitation, discuss emerging/potential approach
getting good spectra,maldi, esi techniques
Resolution, sensitivity
instrument resolution needed, software features to help process this type of data
analytical methods to describe specificity
1) platform development to understand ADC stability in vivo2) ADC catabolism3) ADC characterization for le
Metabolites analysis, Quantitation of conjugate species
specific site of conjugation reaction on protein
Inlet approaches - SEC/RPOptimal site characterization approaches (top-down/bottom- up/middle...etc.)MS
Discussion of different top-down and bottom-up approaches to to narrow down the site of conjugation or met
Assay sensitivity.
a mathematically correct and defendable model for full mass spectral profile mode data on these complex mi
2D chrom, Gel ADC samples and PK profiling,
Software
The challenges of developing SOPs, methods and validations to meet the regulatory requirements from the F
Interested in knowing approaches being used out there.
how do they differentiate from other drugs (mAb, small molecule) in terms of successfully passing through cli
Stability issue; Internal standard
I am here to learn
Insource artifacts like oxidation or dehydration.
Sample workup such as immunocapture; can this conjugates be ditecetd aftre digestion by MS?
sensitivity,resolution and software analysis
I think the largest issue is the stability of the linker that conjugates the toxic payload and how to design it to a
transition from research based experiments to routine (GMP/GMP like) experiments.