

Roundtable: Amino Acid Analysis: Alive and Analyzing

<p>David Chin Chiron Corp. Emeryville, CA 94608 Chair and Moderator</p>	<p>Steven A. Cohen, PhD Director of Proteomics and Life Sciences Technology Waters Corporation Milford, MA 01757 Instrument or Method: Pico·Tag®</p>
<p>Peter Hunziker Univ. of Zurich Biochem Inst. Zurich, SWITZERLAND Instrument or Method: OPA/Fmoc</p>	<p>Dan Strydom Forensic Chemistry & Biology Nebraska Wesleyan University Lincoln, Nebraska Instrument or Method: AccuQ·Tag®</p>
<p>K. Michael Gibson, PhD, FACMG Professor Department of Molecular and Medical Genetics Director, Biochemical Genetics Laboratory Oregon Health & Science University Portland, OR 97201 Instrument or Method: Lithium Citrate/NIN and MS/MS</p>	<p>Richard Thoma Monsanto St Louis, MO 63122 Instrument or Method: Hitachi with comparisons to Beckman 6300</p>
<p>Mark Longster CANCELLED Biochrom Ltd Cambridge, CB4 0FJ England Instrument or Method: Biochrom</p>	

Sensitivity of Systems

	On Column: (in pmole)	pmol	pmol	Injection Volume	In processed sample: uM	uM	uM
	LOD	LOQ	Linear range		LOD	LOQ	Linear Range
Waters/Pickering LiCitrate/NIN	50	250	500-25000	50 uL	1.0	5.0	10 - 500
MS/MS	0.3*	0.6*	3 - 4,000		0.1	0.2	1 - 1265
Hitachi	30	100	100 - 50,000	50 uL	0.6	2.0	2 - 1000
Pico·Tag®	1.0	5.0	20-500	<20 uL	0.05	0.25	1 - 25
AccuQ·Tag®	0.1	.3	0.5 - 100	<20 uL	0.01	0.02	0.03 - 5
AminoQuant II	< 0.5	1	1 - 250	10 uL	0.05	0.1	0.1 - 25

Accuracy and precision under normal conditions

	Accuracy	Precision
Waters/Pickering Beckman LiCitrato	92.8%	6.1%
MS/MS	98.6%	11.8%
Hitachi	96%	4%
Pico·Tag®	95 - 97 %	2 - 8 %
AccuQ·Tag®	97.3%	4%
AminoQuant II	90 - 93 %	1% - 2%

BSA Needed

	BSA hydrolysate (ug)	ug on column	Pmoles BSA on column
Hitachi	1 - 200	0.2 - 40	5 - 1000
Pico·Tag®	0.5 - 50	0.1 - 2	7 - 750
AccuQ·Tag®	0.1 - 10	0.01 - 0.50	1 - 150
AminoQuant II	0.5 - 5	0.02 - 0.30	0.5 - 5

Labor intensity, run times and available automation

	Hydrolysis or Separations	Tech time	Run time	Derivatization Automation	Maintenance Issues
Waters/Pickering LiCitrates	+++	30	140	+	----
OPHL	+	5	2	+	-
Na+ NIN	+++	5	30-120	+	---
Pico·Tag®	+++	50	20	--	-
AccuQ·Tag®	+++	20	50	-	-
AminoQuant II	+++	3	25	+	-

Cost (approximate US Dollars)

Method:	Initial Instrument Cost in US Dollars	Yearly Upkeep:	Tech Time: (in hours)	Consumable Cost per Sample:
Waters/Pickering LiCitrato	80K	4K	0.5	8.15
MS/MS	215K	21K		7.53 (with labor)
Or leased with MC	0	70K		7.53
Hitachi	71K	8K	0.25	\$ Not determined
Biochrom	90K	10K	0.25	\$ Not determined
Pico·Tag®	50K	5K	0.6	3.50
AccuQ·Tag®	50K	5K	0.4	2.50
AminoQuant II	70K	1K	0.25	6.00

Ninhydrin:

Pros:

Almost all physiological amino acid - Ability to Monitor Treatment (Li)

All normal amino acids with Na⁺

Good Specificity

Semi-Automated

Cons:

Slow: LiCitrate over 2 hours

Na⁺ faster

Li⁺ corrosive

Na⁺ “less corrosive” but still high maintenance

Complex system

MS/MS:

Pros:

Fast (2 min analysis)

High sensitivity

Good Specificity

Semi-Automated

Less mechanical

Cons:

Expensive equipment (\$215K, but high through-put)

Lacks isomer separation

Currently Only Arg, Cit, Leu, Phe, Tyr and Met

Pre-Column:

Pros:

Higher sensitivity

Simpler instruments

Turn-on-and-Analyze System

Rapid

Cons:

Sensitive Chemistry - Excipients

Not all the sample is used

Short column life with OPA/Fmoc

Unstable derivatives, except with AccuQ·Tag®

Amino Acid Analysis Research Group

Ongoing members of the AAARG are **David Chin** (chair), **William Lane** (EB liaison), **Anthony Le** and **Johann Schaller**.

Reed Harris and **Peter Hunziker** are stepping down from the AAARG.

We would like to thank them for their valuable contributions during all these years. Special thanks go to **Peter Hunziker** as a longtime chairman of the AAARG.

After the ABRF2004 symposium **Kerry Maddox** AND **Jack Presley** will join the AAARG.

For the continuous rotation of group members we are looking for new members. If you are interested in amino acid analysis and in actively participating in the ABRF please contact **David Chin** (David_Chin@chiron.com). The results of the AAA2001 and AAA2003 studies continue to be available as electronic posters on the ABRF website (www.abrf.org).