ASSESSMENT OF WITHIN-PACKAGE AND LOT-TO-LOT VARIABILITY ASSOCIATED WITH QUARTZ COLLECTION PADS IN THE DETERMINATION OF METALS IN AEROSOL

Presentation ST30

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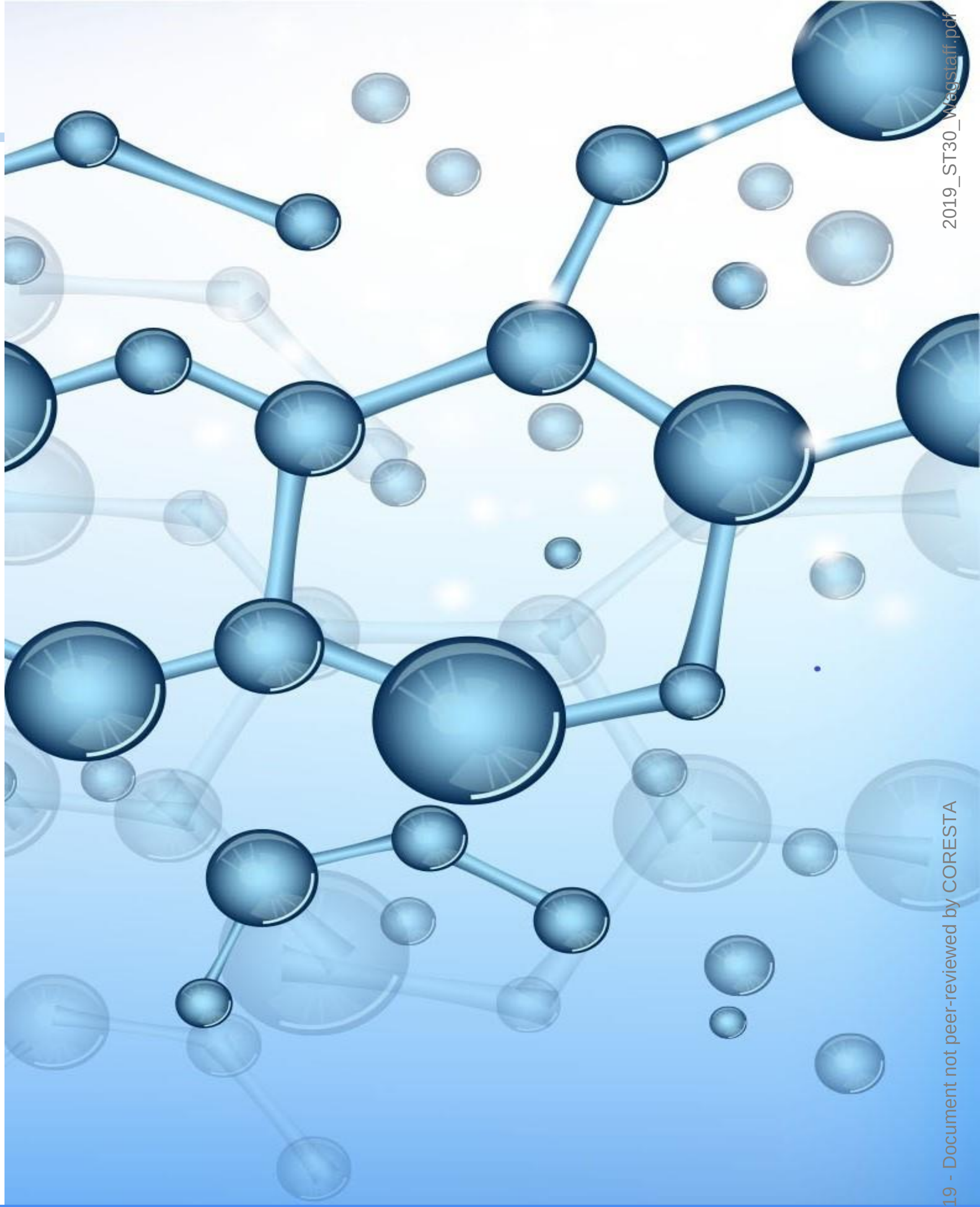


Presentation Outline

- Background and Objectives
- Study Design
  - Analysis of Quartz Pads
  - Statistical Analysis Methodology
- Analytical Results
  - Interpretation of Data
- Summary
  - Future Applications

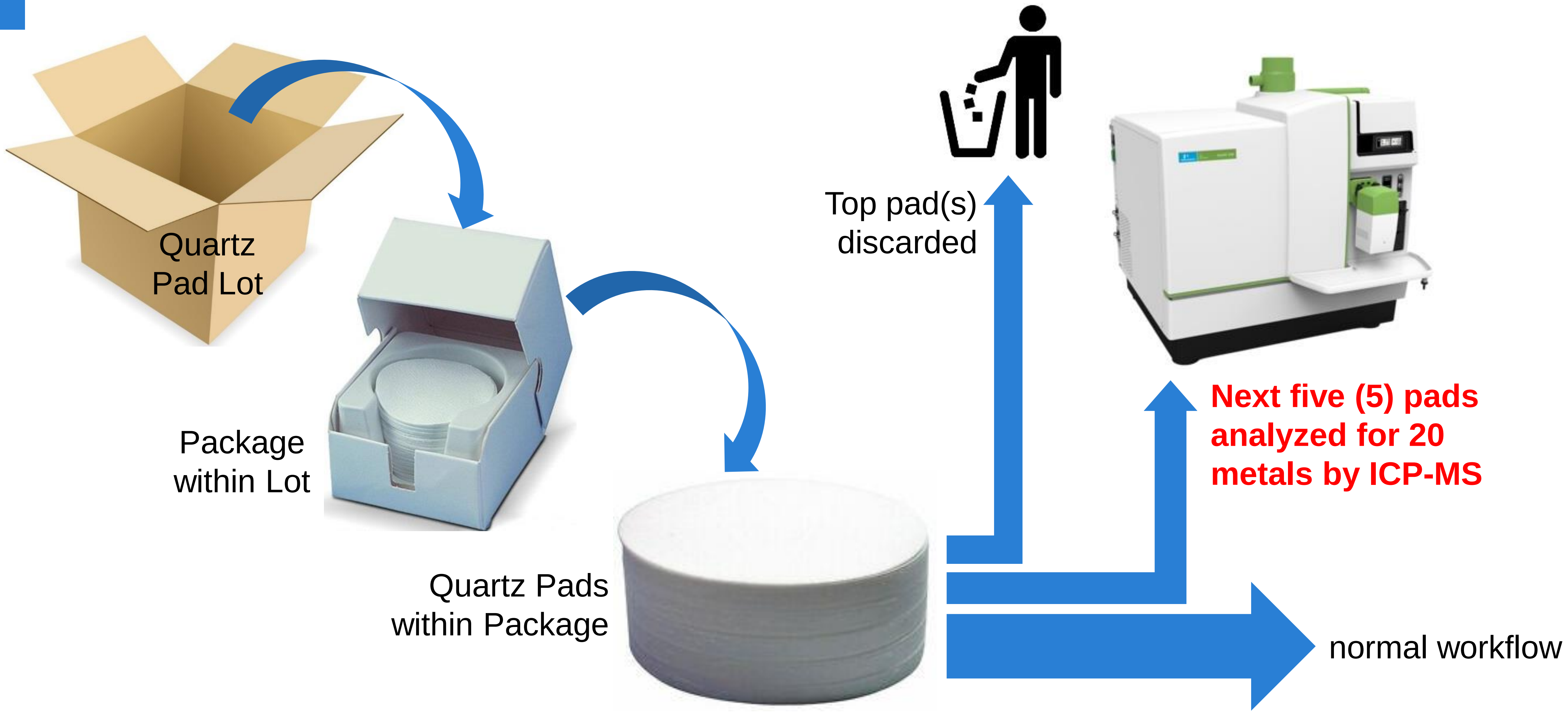
Background – Previous Work

- Continuation of work introduced at 2016 CORESTA Congress
  - *An Alternative Strategy for the Determination of Metals in the Aerosol from Electronic Cigarettes*
- Developed Method for collection of aerosol using quartz pads
  - Pre-washed quartz filter pads, containing no binders, specifically designed for the analysis of metals
  - initial method development work identified factors such as quartz pad **lot** and quartz pad **package** within lot as potential factors affecting overall method variability
  - identified **location** of quartz pad within package (i.e. close to packing material) as a potential contributor of background metals contamination

Objectives

- Quantify levels of 20 metals in untreated quartz pads from various **lots** and **packages** over an extended period of time
- Perform statistical analysis on the data
  - Quantify lot-to-lot variability, package-to-package variability and within-package variability for each of 20 metals
- Use statistical analysis findings to assess potential limitations in determination of differences between metals measured in ENDS product aerosols and collection (air) blanks

Study Design – Analysis of Untreated Quartz Pads



Study Design – Statistical Analysis Methodology

- Data collection occurred over a 1+ year period
- Statistical Analysis: Multifactor Analysis of Variance (ANOVA)
  - Factors: (1) quartz pad lot and (2) quartz pad package within lot

    LOT = {1 (R7CA61558), 2 (R7HA53231), ..., 10 (R8PA50484)}

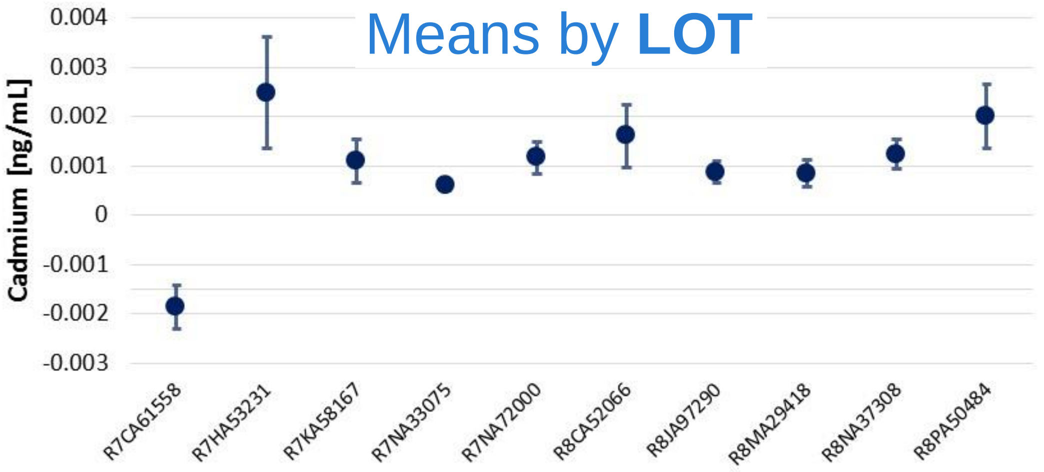
    PACKAGE = {1, 2, 3, … ,190}

    5 replicate pads/pack x 20 metals analytes ~ 19,000 data points

Lot 1 (R7CA61558)				**Lot 2 (R7HA53231)**				**…**			**Lot 10 (R8PA50484)**			
Pack 1	Pack 2	Pack 3	Pack 4	Pack 5	Pack 6	…	Pack 24	…	…	…	…	Pack 188	Pack 189	Pack 190

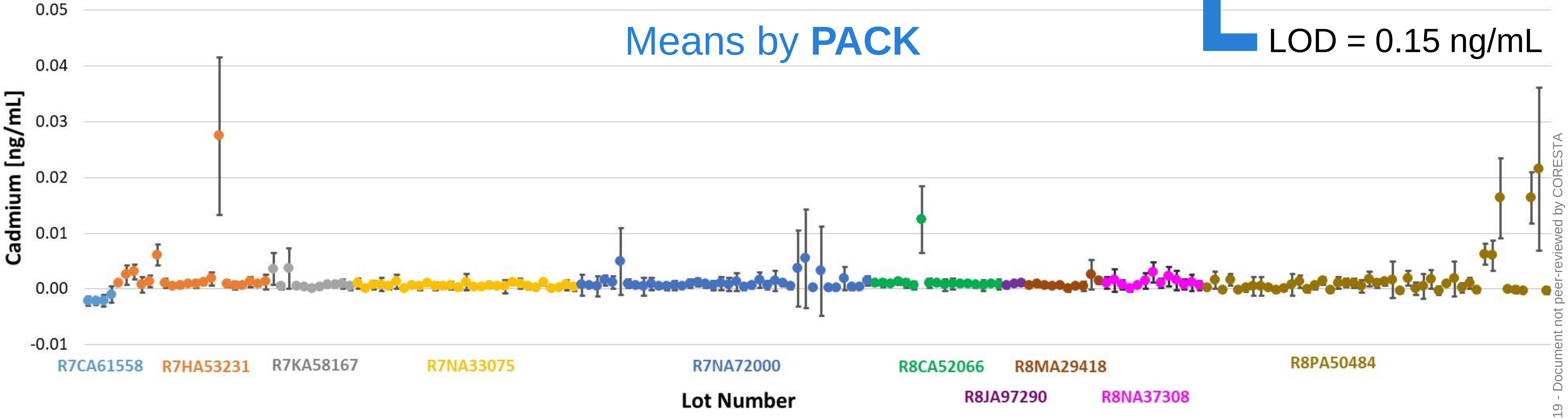
  - Since each lot has its own unique set of packages, PACK is a nested factor within LOT, designated as PACK(LOT) in all results

Analytical Results – Cadmium (Mean ± 95% C.I.)

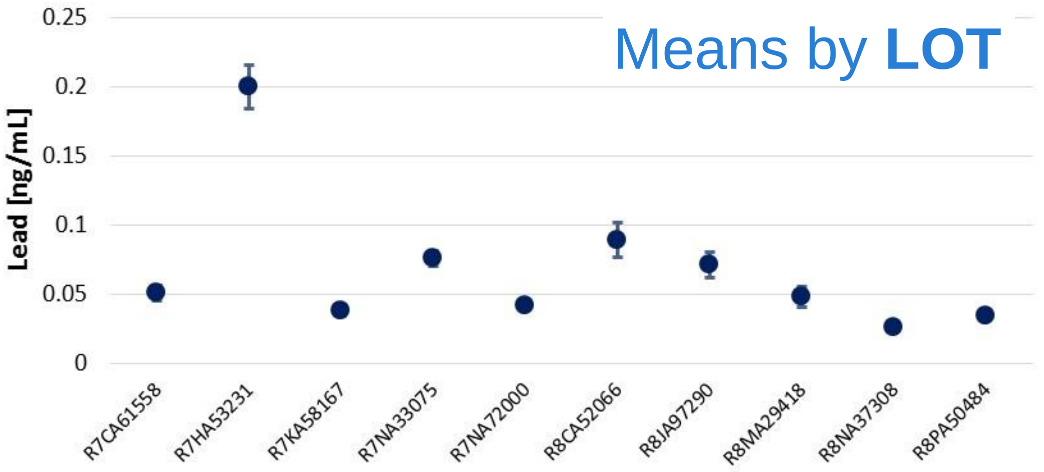


Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
LOT	9	0.0005	0.000061	15.9	<.0001	4.8%	
PACK(LOT)	180	0.0078	0.000044	11.4	<.0001	69.4%	
Residual	759	0.0029	0.000004			25.7%	0.002

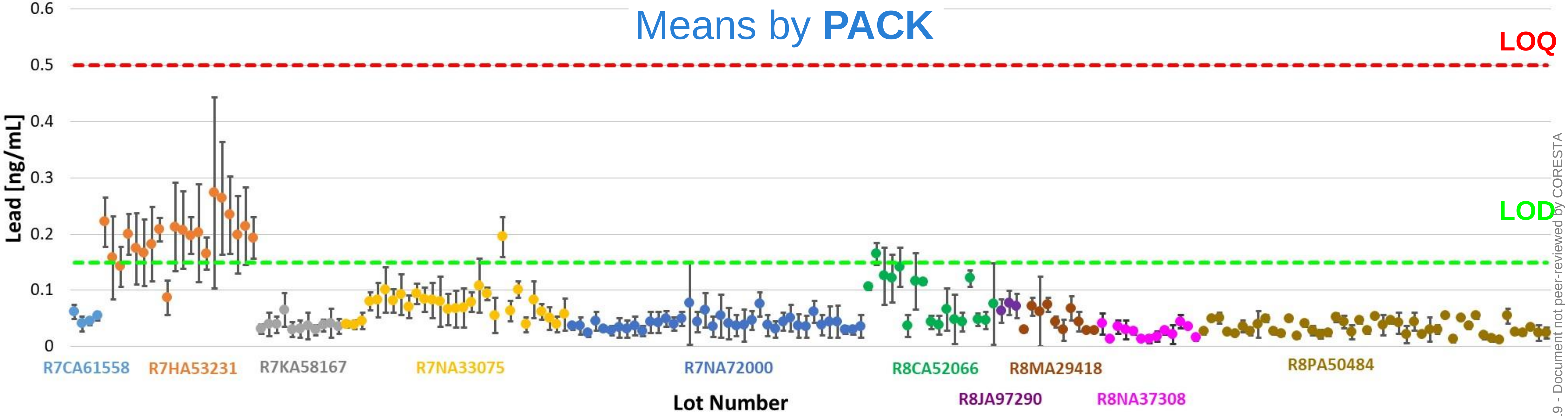
LOD = 0.15 ng/mL



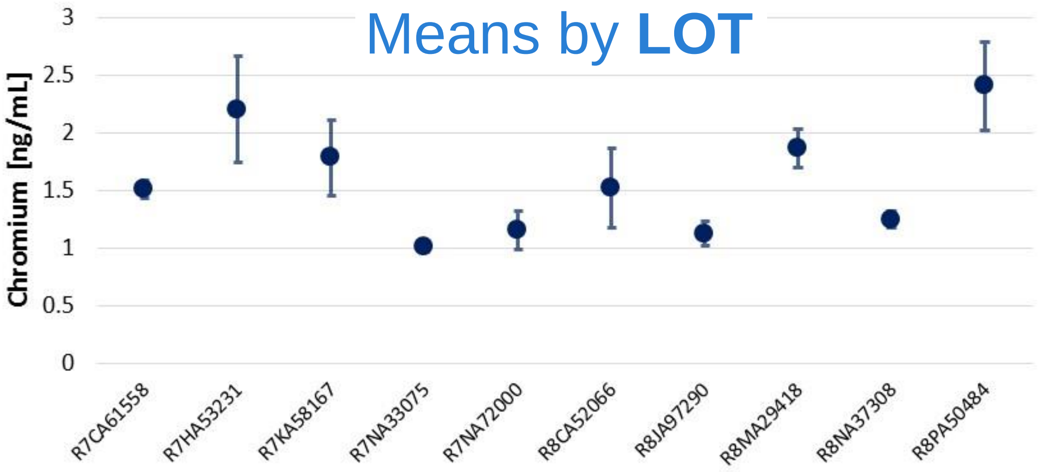
Analytical Results – Lead (Mean ± 95% C.I.)



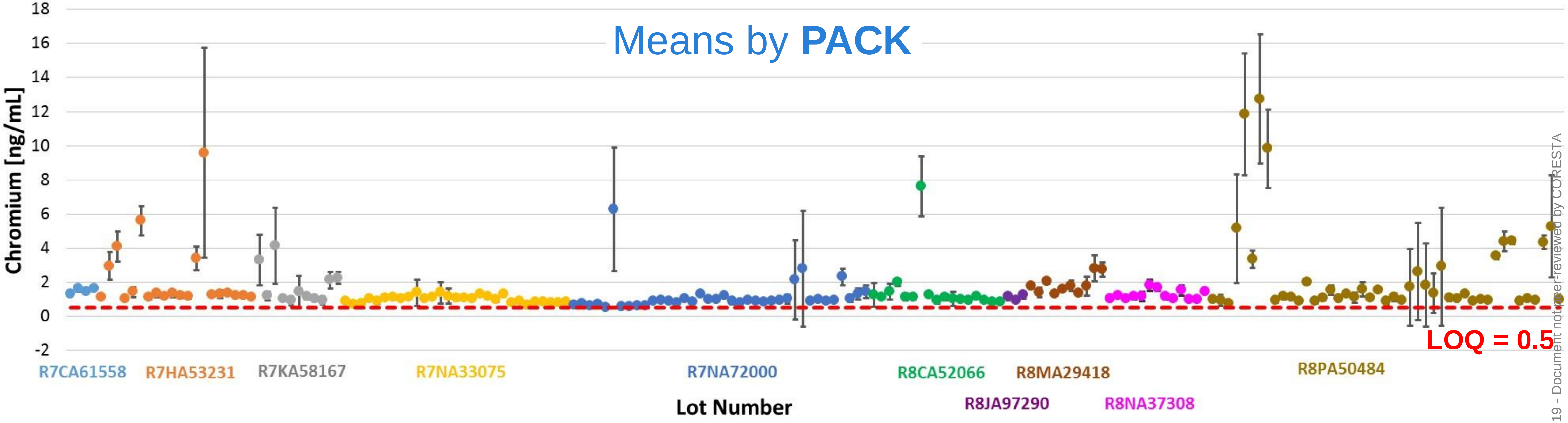
Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
LOT	9	2.37	0.263	282	<.0001	64.4%	
PACK(LOT)	180	0.602	0.003	3.59	<.0001	16.4%	
Residual	760	0.708	0.0009			19.3%	0.031



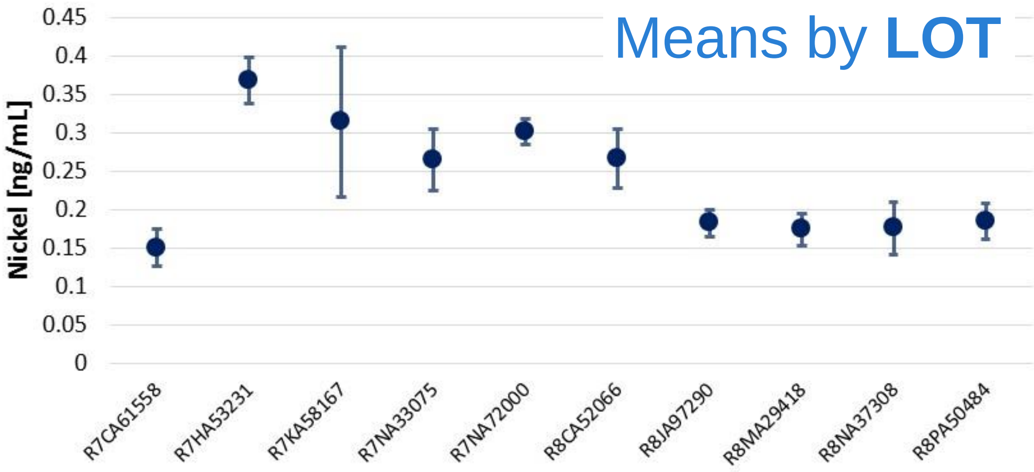
Analytical Results – Chromium (Mean ± 95% C.I.)



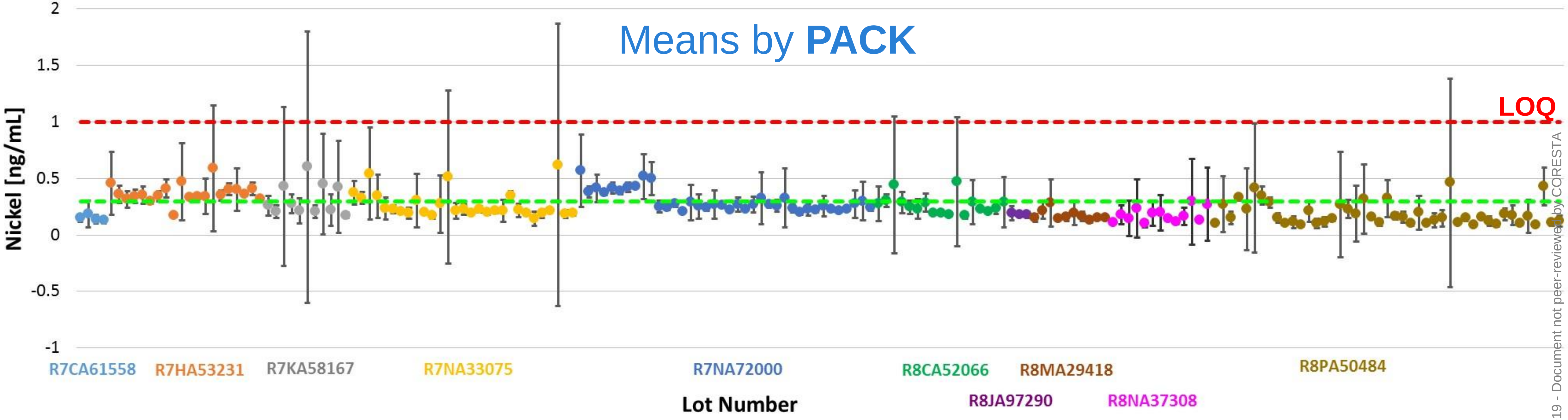
Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
LOT	9	285.7	31.7	53.6	<.0001	8.6%	
PACK(LOT)	180	2572	14.3	24.1	<.0001	77.8%	
Residual	760	450	0.592			13.6%	0.770



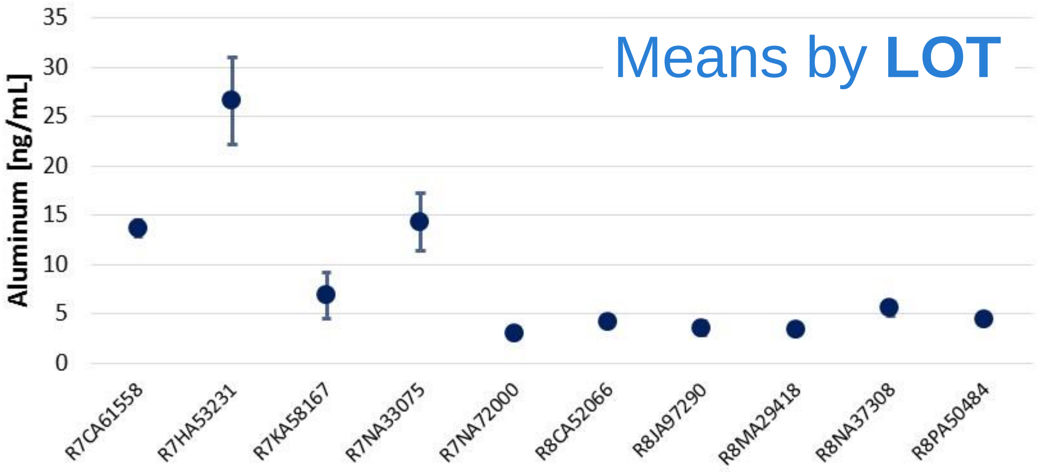
Analytical Results – Nickel (Mean ± 95% C.I.)



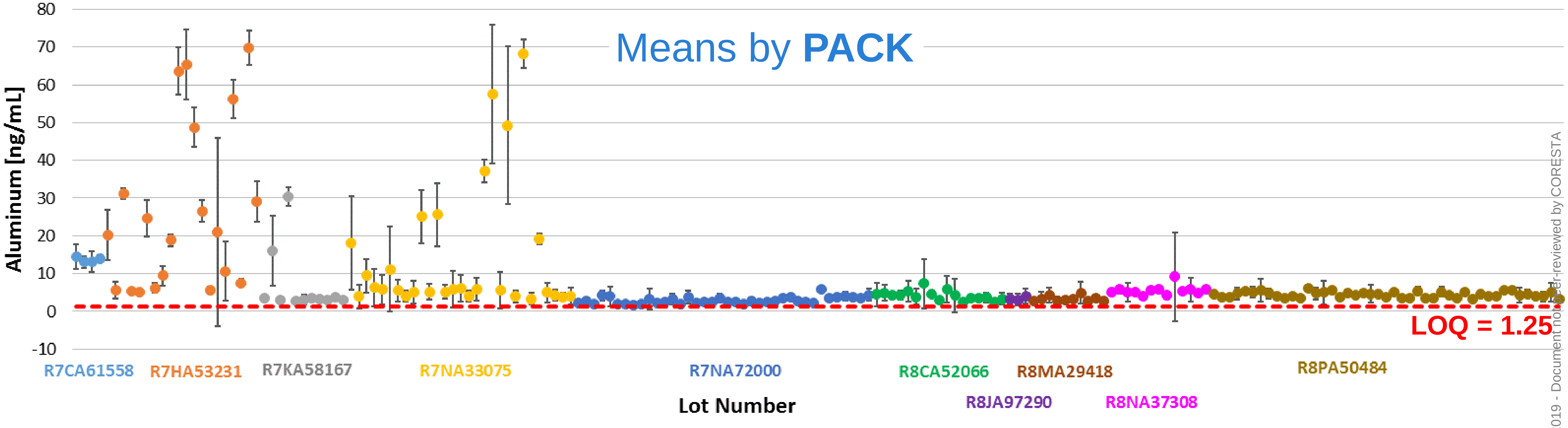
Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
LOT	9	4.07	0.452	14.5	<.0001	11.3%	
PACK(LOT)	180	8.18	0.045	1.46	0.0004	22.8%	
Residual	760	23.7	0.03			65.9%	0.177



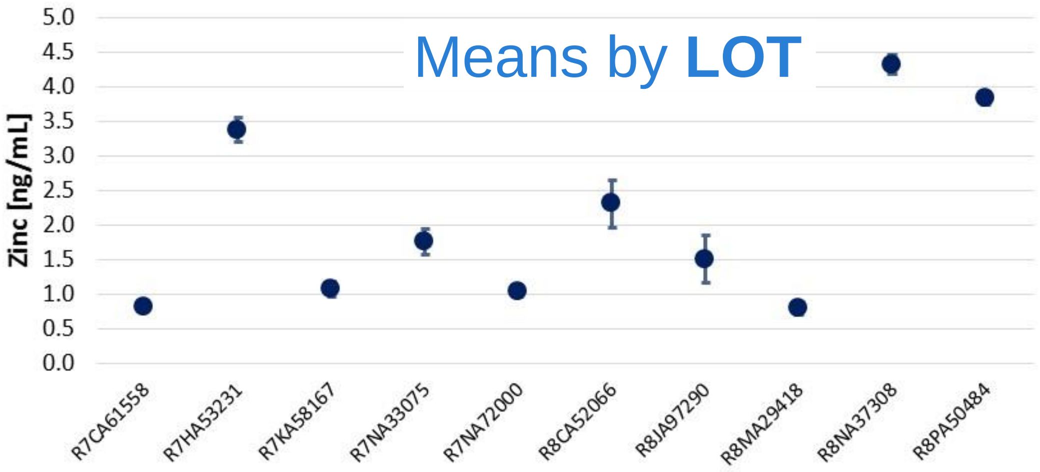
Analytical Results – Aluminum (Mean ± 95% C.I.)



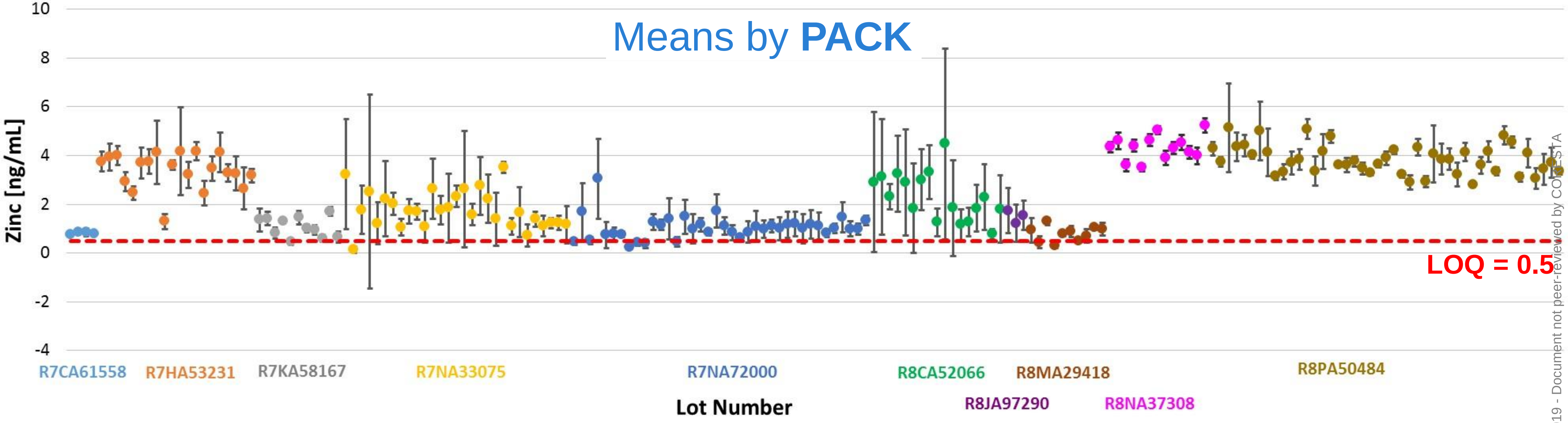
Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
LOT	9	51563	5729	581.4	<.0001	33.7%	
PACK(LOT)	180	93971	522	52.98	<.0001	61.4%	
Residual	759	7479	9.85			4.9%	3.14



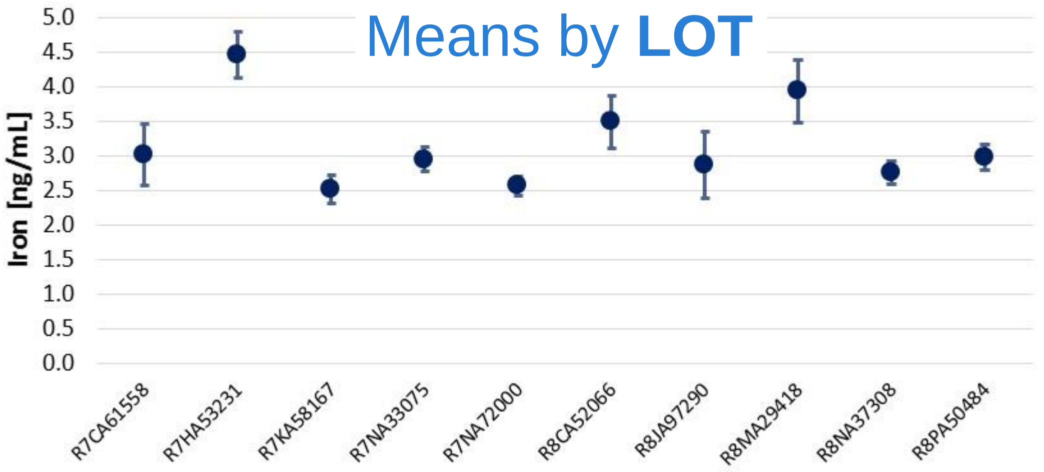
Analytical Results – Zinc (Mean ± 95% C.I.)



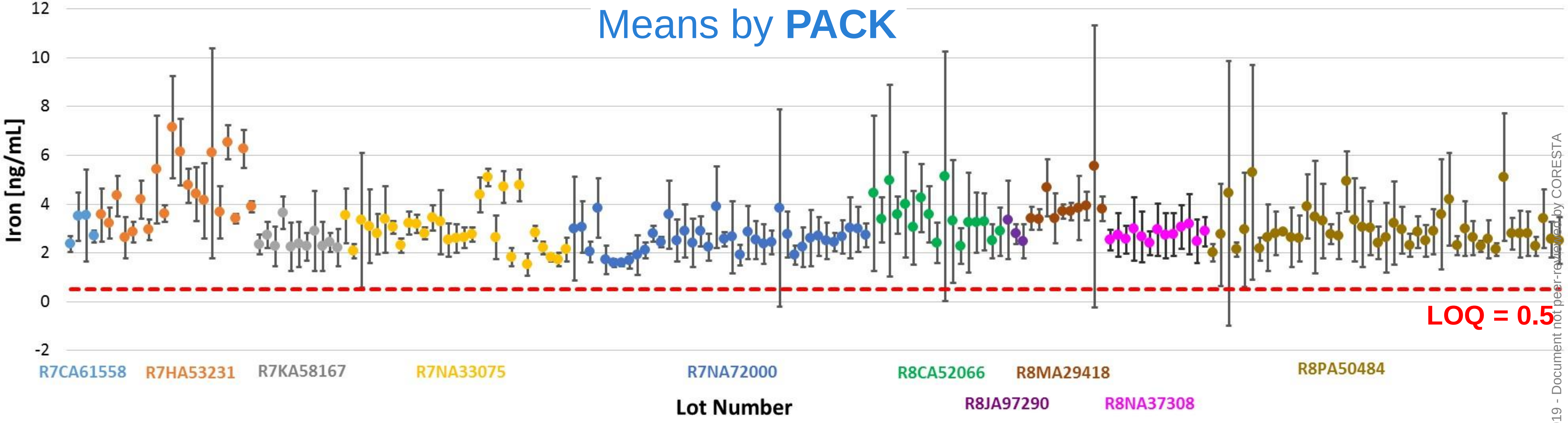
Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
LOT	9	1501.6	166.8	384.5	<.0001	68.6%	
PACK(LOT)	180	358.1	1.99	4.59	<.0001	16.4%	
Residual	757	328.4	0.434			15.0%	0.659



Analytical Results – Iron (Mean ± 95% C.I.)



Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
LOT	9	319.0	35.4	28.3	<.0001	17.2%	
PACK(LOT)	180	582.7	3.24	2.59	<.0001	31.5%	
Residual	758	948.4	1.25			51.3%	1.12



Analytical Results – Remaining Metals

Analyte	Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
Selenium	LOT	9	0.014	0.00153	107.5	<.0001	24.0%	
	PACK(LOT)	180	0.033	0.00018	12.8	<.0001	57.2%	
	Residual	760	0.011	0.00001			18.8%	0.004
Silver	LOT	9	0.0003	0.000033	7.12	<.0001	4.9%	
	PACK(LOT)	180	0.0022	0.000012	2.70	<.0001	37.1%	
	Residual	759	0.0035	0.000005			58.0%	0.002
Tin	LOT	9	5.16	0.573	200	<.0001	26.3%	
	PACK(LOT)	180	12.3	0.068	23.9	<.0001	62.7%	
	Residual	759	2.17	0.003			11.1%	0.053
Tungsten	LOT	9	0.63	0.070	40.0	<.0001	6.1%	
	PACK(LOT)	180	8.46	0.047	26.7	<.0001	81.1%	
	Residual	760	1.34	0.002			12.8%	0.042

Se < LOD (0.024 ng/mL)

Ag < LOD (0.15 ng/mL)

Sn < LOD (0.15 ng/mL)

W < LOD (0.15 ng/mL)

Analytical Results – Remaining Metals

Analyte	Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
Beryllium	LOT	9	0.00013	0.0000143	66.2	<.0001	27.8%	
	PACK(LOT)	180	0.00017	0.0000010	4.37	<.0001	36.6%	
	Residual	760	0.00017	0.0000002			35.6%	0.0005
Titanium	LOT	9	1.46	0.162	40.1	<.0001	23.4%	
	PACK(LOT)	180	1.69	0.009	2.33	<.0001	27.3%	
	Residual	760	3.06	0.004			49.3%	0.064
Manganese	LOT	9	1.59	0.177	25.2	<.0001	13.0%	
	PACK(LOT)	180	5.40	0.030	4.27	<.0001	43.9%	
	Residual	756	5.31	0.007			43.2%	0.084
Cobalt	LOT	9	0.002	0.00023	9.27	<.0001	7.3%	
	PACK(LOT)	180	0.008	0.00004	1.68	<.0001	26.4%	
	Residual	759	0.019	0.00003			66.3%	0.005
Copper	LOT	9	0.355	0.039	8.59	<.0001	6.7%	
	PACK(LOT)	180	1.47	0.008	1.78	<.0001	27.7%	
	Residual	757	3.47	0.005			65.6%	0.068

Be < LOD (0.003 ng/mL)

Ti < LOQ (0.625 ng/mL)

Mn < LOQ (0.5 ng/mL)

Co < LOD (0.03 ng/mL)

Cu < LOD (0.188 ng/mL)

Analytical Results – Remaining Metals

Analyte	Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
Zirconium	LOT	9	1.15	0.1276	929	<.0001	41.7%	
	PACK(LOT)	180	1.50	0.0084	60.8	<.0001	54.5%	
	Residual	760	0.104	0.0001			3.8%	0.012
Molybdenum	LOT	9	911	101	37.5	<.0001	3.9%	
	PACK(LOT)	178	20596	116	42.8	<.0001	87.5%	
	Residual	752	2031	2.70			8.6%	1.64
Strontium	LOT	9	20.6	2.29	716	<.0001	34.1%	
	PACK(LOT)	180	37.4	0.208	64.9	<.0001	61.9%	
	Residual	760	2.43	0.003			4.0%	0.057
Arsenic	LOT	9	5.40	0.600	48.8	<.0001	9.8%	
	PACK(LOT)	180	40.2	0.223	18.2	<.0001	73.2%	
	Residual	758	9.32	0.012			17.0%	0.111

Zr is **quantifiable** (LOQ = 0.1)

Mo is **quantifiable** (LOQ = 0.25)

Sr is *occasionally* **quantifiable** (LOQ = 0.25)

As is *occasionally* **quantifiable** (LOQ = 0.15)

Analytical Results – Interpretation of Data

- Clearly, both quartz pad lot *and* quartz pad package within lot are significant factors affecting yields for all 20 tested metals
- Zinc and lead show highly significant LOT effects, where the mean for *all* packages within a lot are removed from the majority of other lots and packages
- About half the tested metals show highly significant PACK(LOT) effects (e.g. aluminum, chromium, arsenic, molybdenum)
- For the remaining eight metals (e.g. iron, nickel, silver, copper), while both lot and package are significant factors, the majority of the variance is still unaccounted for in these 2 factors

Analytical Results – Interpretation of Data

- Strategies must be adopted to ensure quartz pads from the <u>same package</u> are used in generating samples for both ENDS product aerosols and associated collection (air) blanks
- Such a strategy will eliminate the clear influence of both LOT and PACK(LOT) effects on the levels of background metals in untreated pads prior to their use in sample collection, leaving only within-package sources of variation

Analytical Results – Interpretation of Data

Analyte	Source	DF	Sum of Squares (SS)	Mean Square	F-Value	Pr. > F	SS Ratio (%)	Res. SD (ng/mL)
Iron [ng/mL]	LOT	9	319.0	35.4	28.3	<.0001	17.2%	
	PACK(LOT)	180	582.7	3.24	2.59	<.0001	31.5%	
	Residual	758	948.4	1.25			51.3%	1.12

- Within-package variation for levels of background metals on untreated pads can be estimated by the ANOVA residual mean square (i.e. pooled within-package variance)

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= pooled within-package variance

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Analytical Results – Interpretation of Data

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= pooled within-package variance

$\sqrt{MSRes}$ = pooled within-package standard deviation

- Within-package variation for levels of background metals on untreated pads can be estimated by the ANOVA residual mean square (i.e. pooled within-package variance)

Analytical Results – Interpretation of Data

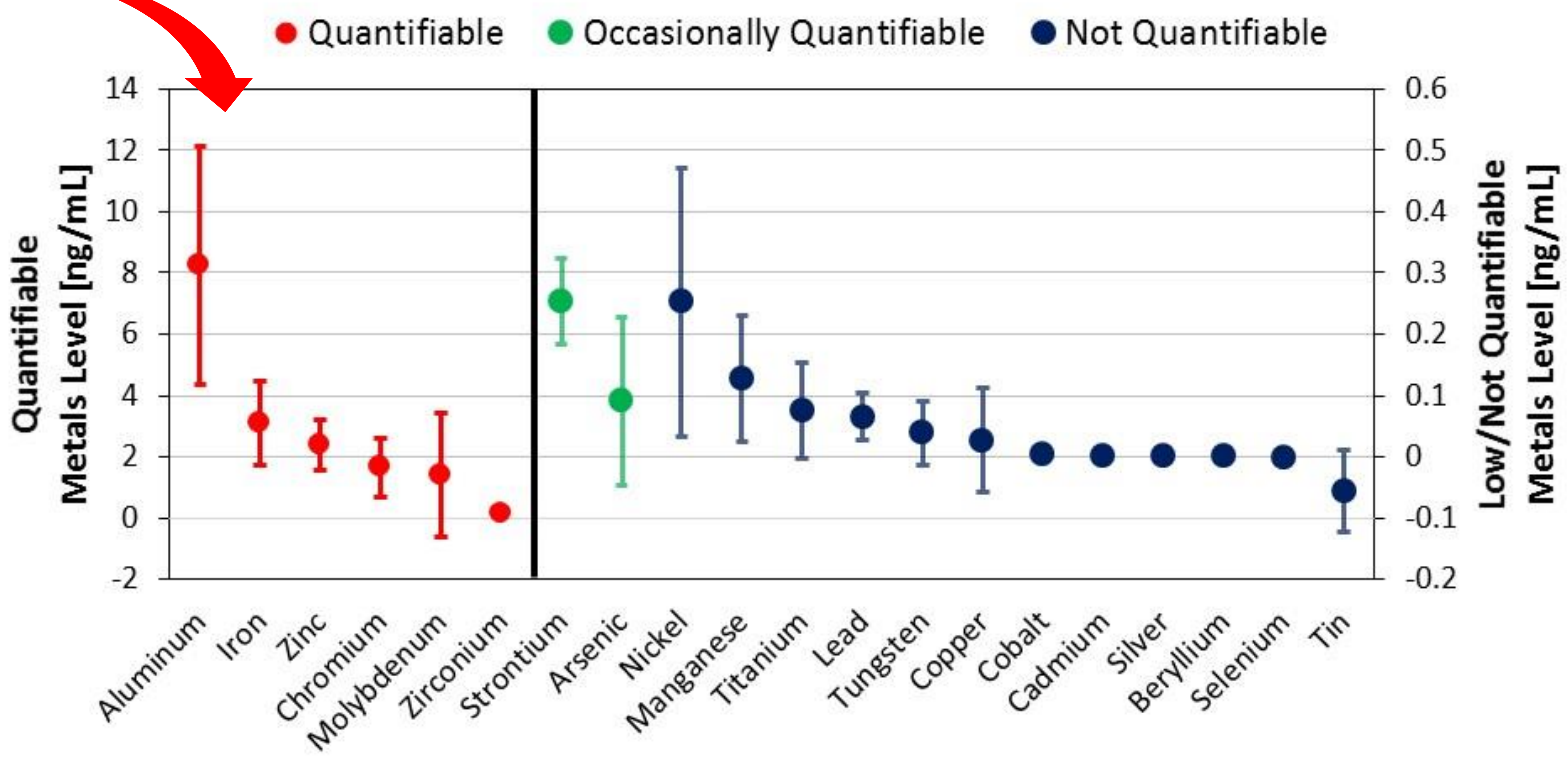
- Although quartz pad lot and package were both significant, the absolute level of each metal also needs to be considered
- Only 6 of 20 metals had ≥50% quantifiable results, with another 2 metals occasionally quantifiable

Metal	Grand Mean (ng/mL)	LOD (ng/mL)	LOQ (ng/mL)	Percent > LOQ	Quantifiable?
Beryllium	0.0003	0.003	0.01	0%	not quantifiable
Aluminum	8.26	0.375	1.25	100%	**quantifiable**
Titanium	0.076	0.188	0.625	0.6%	not quantifiable
Chromium	1.66	0.15	0.5	100%	**quantifiable**
Manganese	0.127	0.15	0.5	1.2%	not quantifiable
Iron	3.10	0.15	0.5	100%	**quantifiable**
Cobalt	0.004	0.03	0.1	0%	not quantifiable
Nickel	0.252	0.3	1	1.1%	not quantifiable
Copper	0.027	0.188	0.625	0.3%	not quantifiable
Zinc	2.38	0.15	0.5	95%	**quantifiable**
Arsenic	0.091	0.045	0.15	12%	**occasionally quantifiable**
Selenium	-0.002	0.024	0.08	0%	not quantifiable
Strontium	0.253	0.075	0.25	18%	**occasionally quantifiable**
Zirconium	0.132	0.03	0.1	85%	**mostly quantifiable**
Molybdenum	1.41	0.075	0.25	50%	**mostly quantifiable**
Silver	0.0004	0.023	0.075	0%	not quantifiable
Cadmium	0.001	0.015	0.05	0%	not quantifiable
Tin	-0.056	0.3	1	0%	not quantifiable
Tungsten	0.039	0.03	0.1	1.9%	not quantifiable
Lead	0.066	0.03	0.1	0.1%	not quantifiable

Analytical Results – Interpretation of Data

- Within-package 95% confidence intervals for levels of background metals on untreated pads as estimated by the ANOVA residual mean square

within-package variation of background levels on untreated pads could have future applications as a "minimum" threshold for identifying differences between ENDS product aerosols and blanks



Summary

- Untreated quartz pads from various lots and packages within lots were analyzed for 20 metals over a 1+ year time frame, resulting in 19,000 data points
- Both LOT and PACK(LOT) were identified as significant factors affecting levels of all 20 tested metals
  - Of the 20 metals, only 6 had reasonably quantifiable levels, with aluminum, iron, zinc, chromium, molybdenum most abundant
- Ensure quartz pads are sourced from a single package
- Within-pack variation of quantifiable metals on untreated pads used as a threshold to identify differences between ENDS aerosol and blanks collected on pads from the same package

Summary – Future Applications

- ENDS aerosol and associated aerosol blanks
  - quartz collection pads are sourced from the <u>same</u> package
  - ENDS aerosol and blanks are from a single analytical run
  - metals analyte is quantifiable in both ENDS aerosol and blank
  - metals analyte is quantifiable on the untreated quartz pads
- Z-Score for each ENDS aerosol observation?

$$\frac{(X_{ENDS} - \bar{X}_{Blank})}{\sqrt{MSRes}}$$

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- Thunder Zheng

THANK YOU !