

Supplementary Material

Harmonization of Rapid Evaporative Ionization Mass Spectrometry Workflows across Four Sites and Testing Using Reference Material and Local Food-Grade Meats

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Supplementary Figure S1: REIMS System set-up and suitability checklist.

1. General

- ☐ Make sure the instrument has been in operate mode for 30 minutes
- ☐ If there is no signal on the instrument when solvent is flowing, try the following in the given order, and check if there is signal after each step:
 - Reinitialise
 - Change to other polarity mode (+) and back
 - Put MassLynx into instrument standby (red), then switch to operate again
 - Restart electronics

Instrument Settings:

- ☐ The correct .ipr file is loaded
- ☐ Negative ionization mode is selected
- ☐ Sensitivity mode is selected
- ☐ Collision gas is ON
- ☐ Collision Energy is OFF
- ☐ Correct scan time is set (1s)
- ☐ Backing pressure is >1.5
- ☐ Collision pressure is between 1.5×10^{-1} – 2.5×10^{-1}
- ☐ TOF pressure is below 1.1×10^{-6} , but ideally below 9×10^{-7}
- ☐ Check the following instrument parameters are set, when MS Profile Type is set to Auto P, the grey values don't matter anymore:

Sampling Cone	40	REIMS tab
Source Offset	80	REIMS tab
Collision RF Offset	150	Setup/RF settings
Collision RF Initial	50	
Collision RF Final	150	
MS Profile Type	Auto P	Setup/Quad profile
MSProfileMass1	100	
MSProfileDwellTime1	20	
MSProfileRampTime1	20	
MSProfileMass2	300	
MSProfileDwellTime2	20	

2. REIMS source parameters:

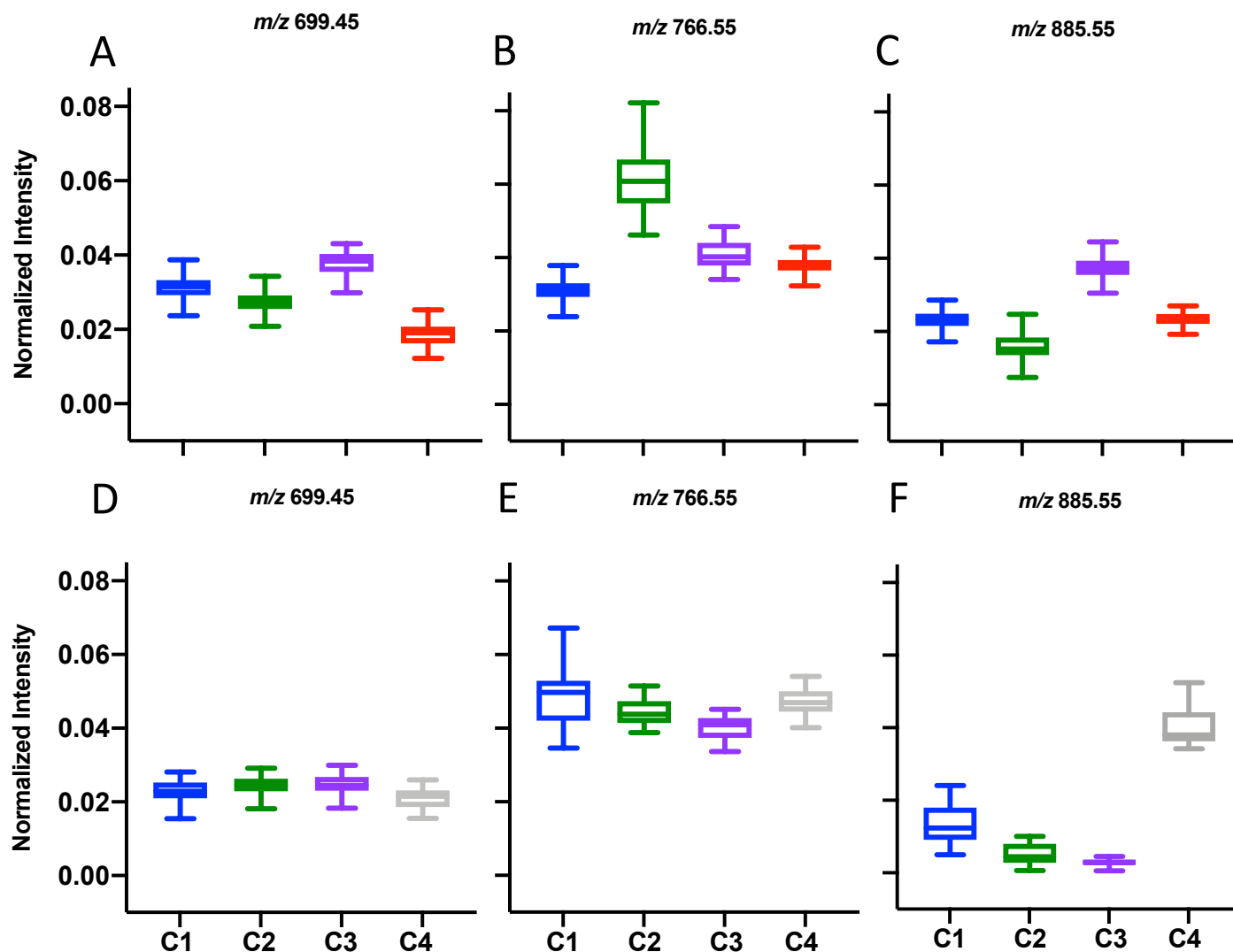
- ☐ Heater is turned ON
- ☐ Small IPA capillary is in place
- ☐ The impactor holder assembly is open
- ☐ After starting leu-enk in IPA, leu-enk peak is visible around 554.2
- ☐ There is no peak splitting of 554.2
- ☐ Check the resolution is >15000 (for this use ResCalc software)
- ☐ Correct mass range is set (usually 50-1200)
- ☐ Check if the file duration is set to 0 when starting a file

Routine REIMS checklist:

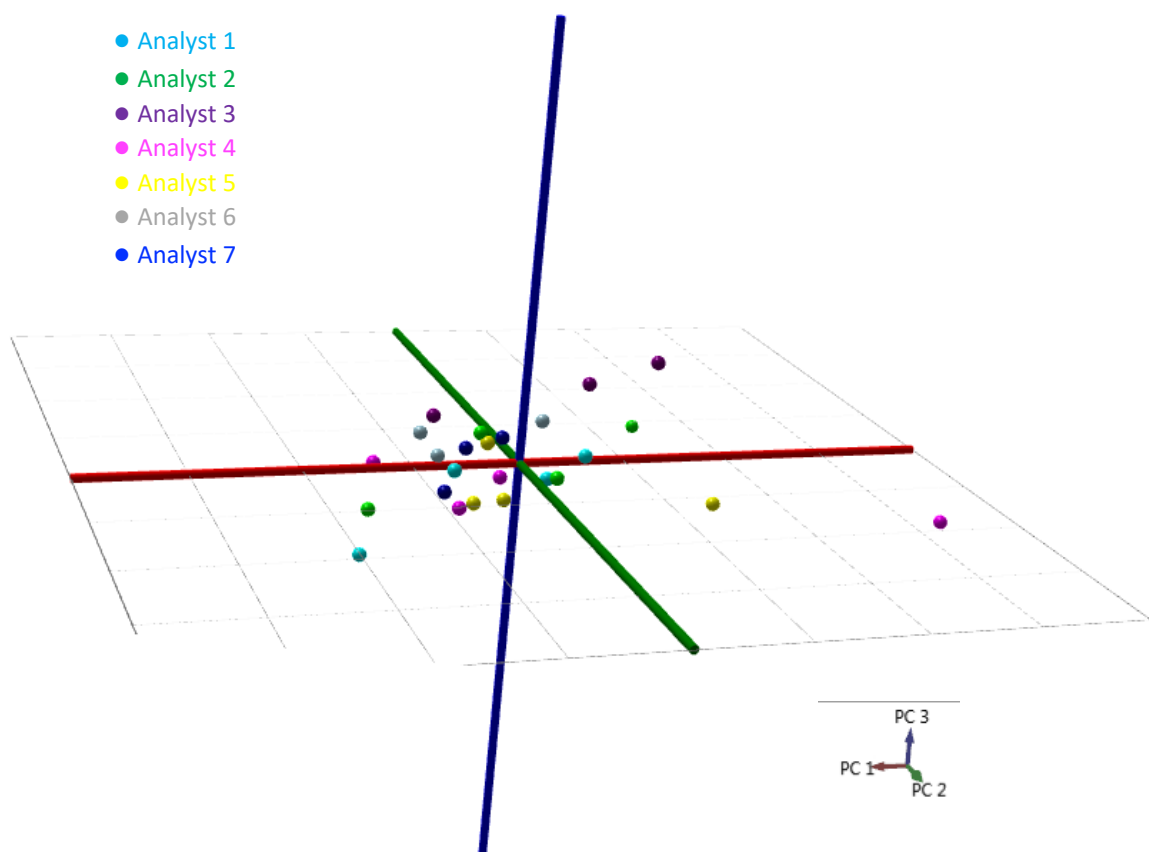
- ☐ The instrument has been calibrated/calibration was checked that day
- ☐ The instrument has been calibrated since last electronics shutdown/reboot
- ☐ The detector was calibrated in the past month

***Ex-vivo* breast tissue checklist:**

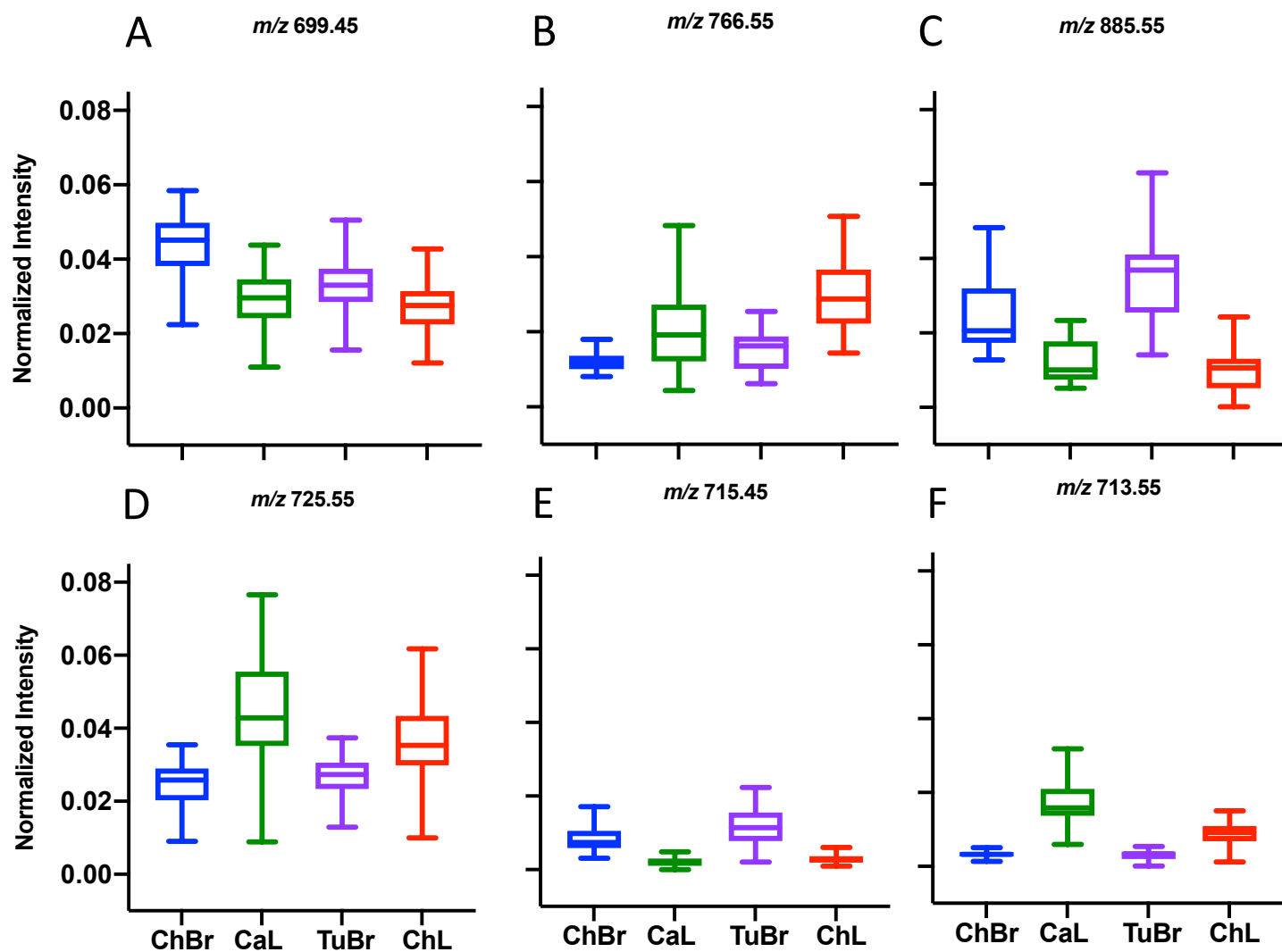
- ☐ The overall TIC (during burns) $> 5e^7$
- ☐ Leu-enk intensity level per scan $1e^5 - 5e^6$ at the beginning
- ☐ Leu-enk intensity level per scan $1e^5 - 5e^6$ at the end
- ☐ Tissue intensity level per scan (highest peak in the m/z 600-1000 range) $> 5e^4$
- ☐ Resolution > 15000 at the beginning of analysis
- ☐ Resolution > 15000 at the end of analysis



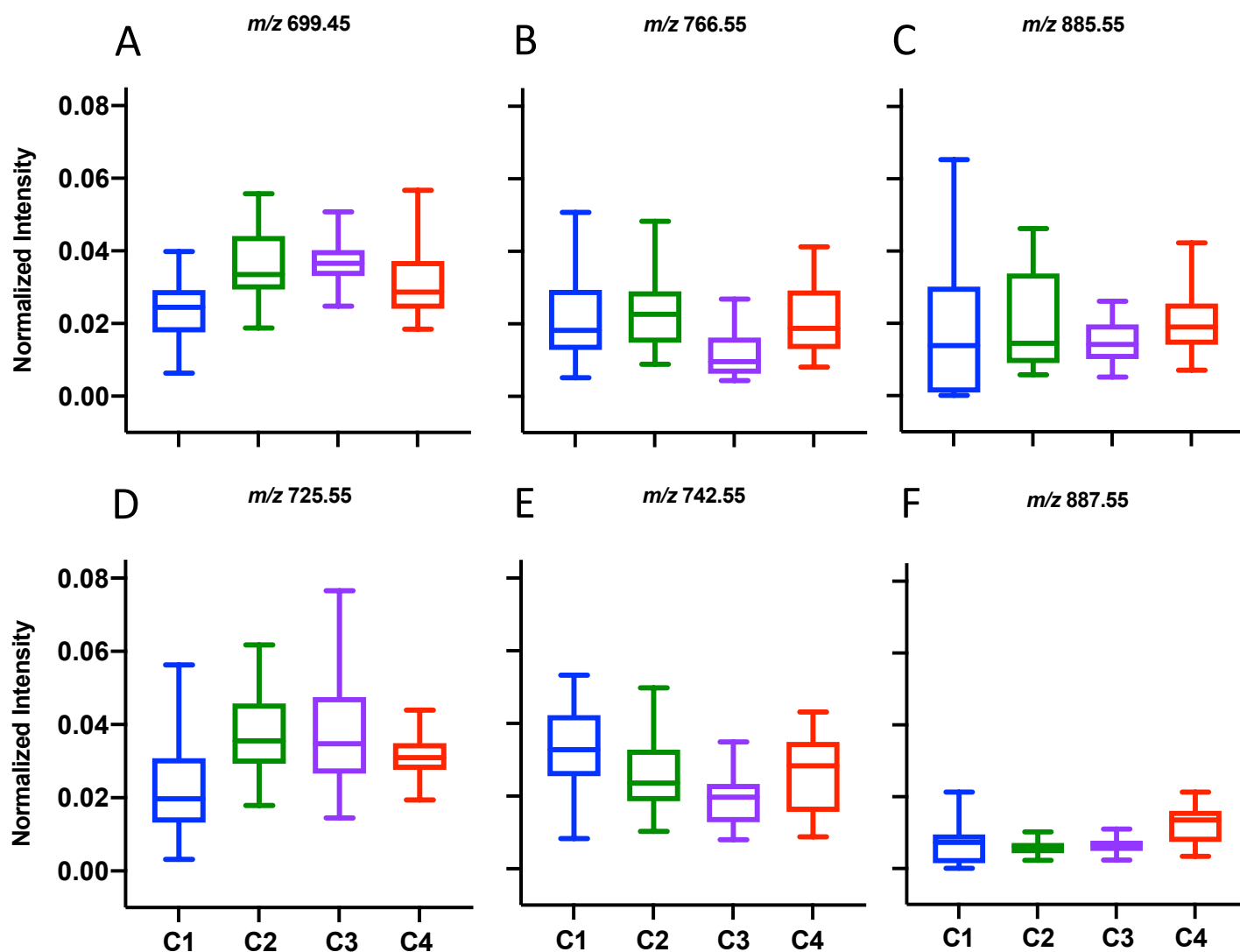
Supplementary Figure S2: Relative abundance of selected m/z bins in pork liver reference material by center. LSVC was used to identify m/z bins that correctly classified center >95% of the time (Table S1). Panels A-C present boxplots of 3/3 bins that achieved optimal cross-validation prior to method harmonization. Panels D-F show boxplots of the same m/z bins after method harmonization, which are a subset of the 13 m/z bins required to achieve optimal cross-validation by site, after method harmonization. C4 used batch 1 pork liver for all analyses. C1-C3 used batch 1 pork liver prior to method harmonization, and batch 2 after method harmonization. $P < 0.0001$ for all plots by non-parametric ANOVA.



Supplementary Figure S3: Analyst-dependent differences in REIMS spectra. Seven analysts from all four centers conducted at least 3 REIMS analyses on batch 1 pork liver at C3. The PCA plot of the m/z 600-1000 region of the mass spectra revealed insignificant analyst-dependent differences associated with sampling technique.



Supplementary Figure S4: Relative abundance of selected m/z bins in food-grade meats by meat type. LSVC was used to identify m/z bins that correctly classified center >95% of the time (Table S1). Panels A-F present boxplots of 6/14 bins that achieved optimal cross-validation. $P < 0.0001$ for all plots by non-parametric ANOVA. ChBr: Chicken breast. CaL: Calf liver. TuBr: Turkey breast. ChL: Chicken liver..



Supplementary Figure S5: Relative abundance of selected m/z bins in food-grade meats by center. LSVC was used to identify m/z bins that correctly classified all food-grade meats center >95% of the time (Table S1). Panels A-F present boxplots of 6/23 bins that achieved optimal cross-validation. $P < 0.0001$ for all plots by non-parametric ANOVA.

Supplementary Table S1: LSVC analysis of pork liver reference material and food-grade meats

Analysis/Parameter	Pork Liver Ref. ¹	Pork Liver Ref. ¹	Food-grade meat ²	Food-grade meat ²
Class for cross-validation	Center	Center	Meat type	Center
Correct classification rate	97%	98%	84.8%	35%
No. of mass bins	3	13	19	20
Selected <i>m/z</i> bin				642.48
				671.47
				673.48
		682.59		
		695.46		
		697.48	697.48	697.48
	699.5	699.55	699.5	
			700.5	700.5
			705.48	705.48
			713.51	
			715.5	715.5
			721.48	
		723.5	723.5	
		725.51	725.51	725.51
			727.53	
				737.51
				738.51
		742.54		742.54
			743.53	743.53
			744.55	744.55
				747.5
			751.52	751.52
	766.54	766.54	766.54	766.54
		767.54		
			768.55	
		769.53		769.53
			770.57	
			773.53	773.53
		787.48		
		794.57		
				861.55
			863.56	
	885.55	885.55	885.55	
				887.56

¹LSVC analysis optimized to achieve >95% correct classification rate for indicated samples and class, based on 4-fold cross-validation. Selected *m/z* bins are indicated.

²Standard LSVC and cross-validation by leaving one site out (meat type as class) or leaving one meat type out (center as class) results for food-grade meat by meat type or center. Selected *m/z* bins are indicated.