Supplementary Materials for

Detection of titanium dioxide particles in human, animal and infant formula milk

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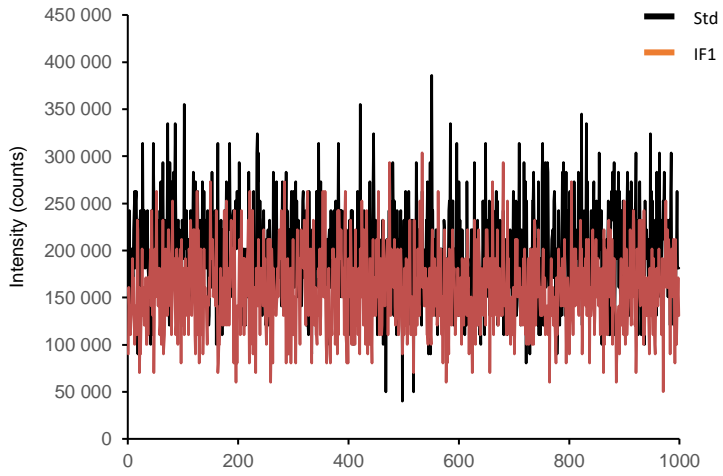
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The PDF file includes:
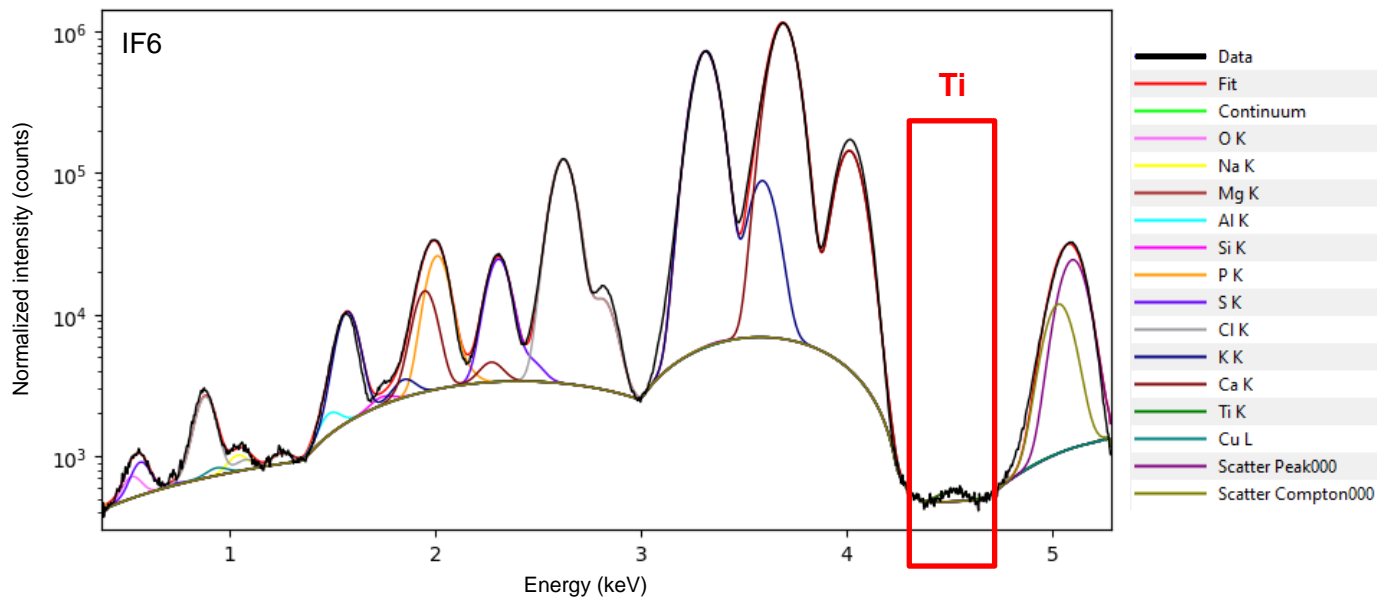
Fig. S1. Acquisition of Ti signal (m/z = 131) of IF1 mineralized milk sample diluted 1:10 by ICP-MS using a dwell time of 100 µs in comparison with a 5 µg/L Ti standard solution. Ti concentration of IF1 measured was 4.42 µg/L.

Fig. S2. X-ray fluorescence spectrum collected at 5.1 keV on infant formula sample IF6 and corresponding fit using PyMCA software.

Fig. S3. X-ray fluorescence spectra at 5.1 keV of human (A,B), IF (C-E), and animal milk (F-H).
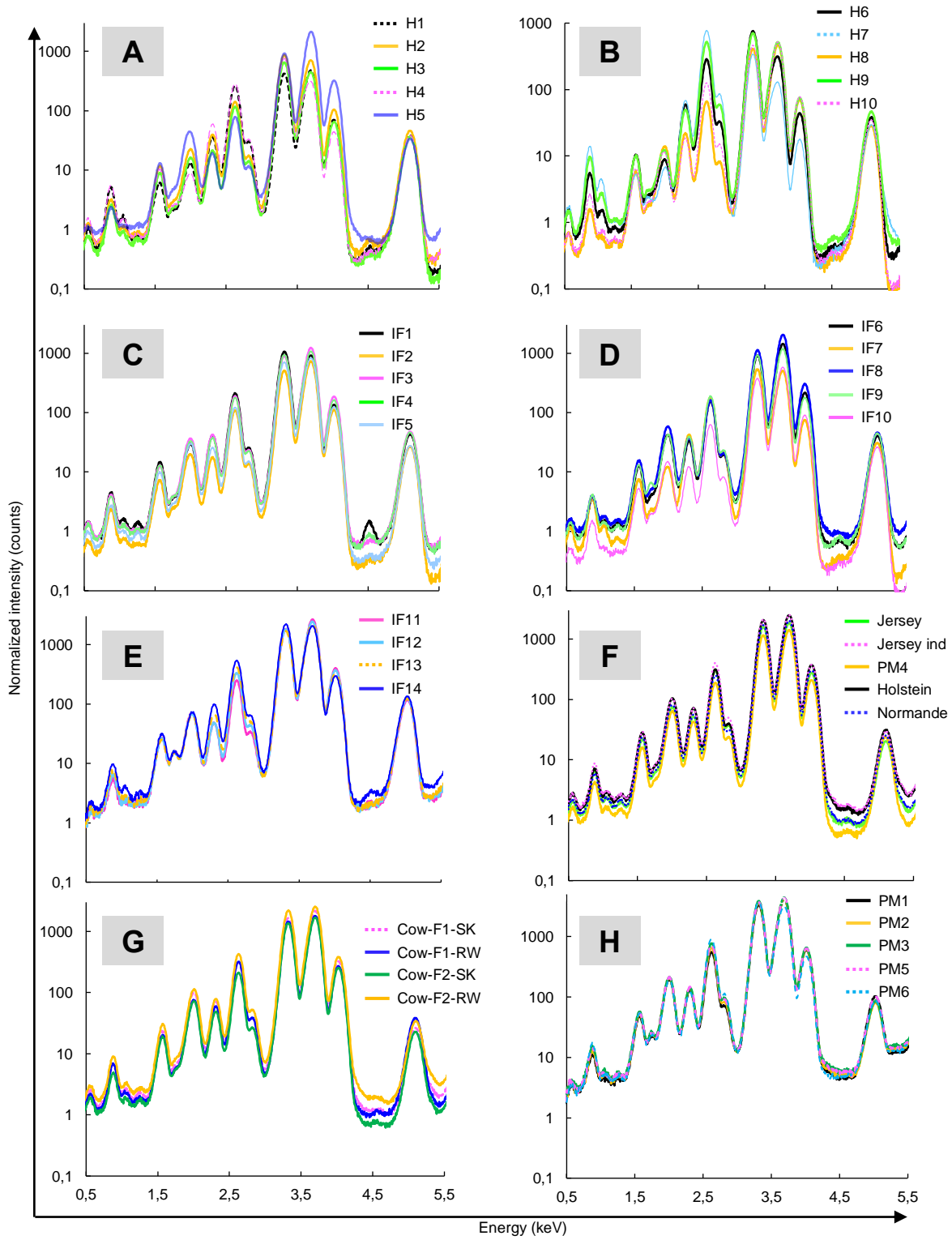
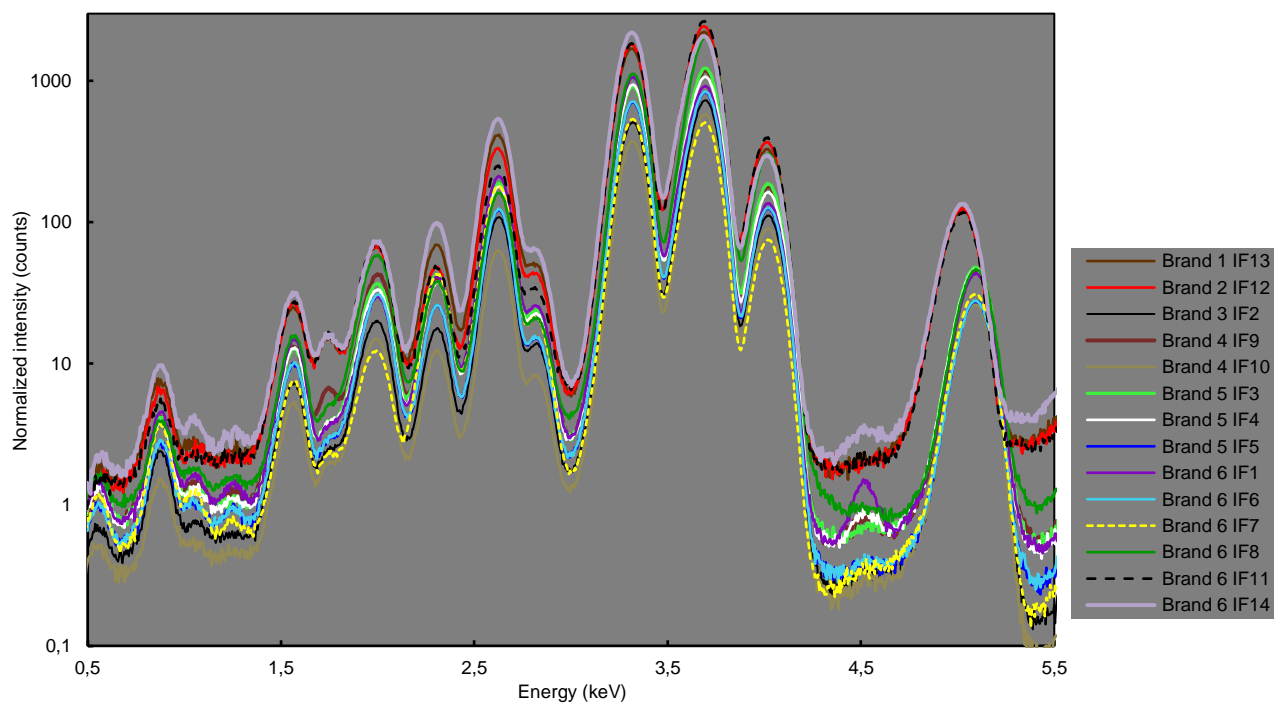**Fig. S4.** X-ray fluorescence spectra collected at 5.1 keV for IF by brand (A). Spectra of different regions of the same pellet IF3 (B), IF4 (C), IF5 (D), and IF7 (E).

Fig. S5. Milk production and quality plotted against Ti AUC in the milk of three breeds of cow. Analysis of milk from Holstein (n=6), Normande (n=6), and Jersey (n=5) cows raised at the INRAE experimental unit. For each breed, individual milk collected on morning milking was blended prior analysis for Ti AUC (red open bars). Ti AUC values were plotted against that of the average per cow of : the volume of milk produced on that day (A), the number of cells per milliliter of milk (B), the protein (C) and fat (D) levels in g per kg of milk.
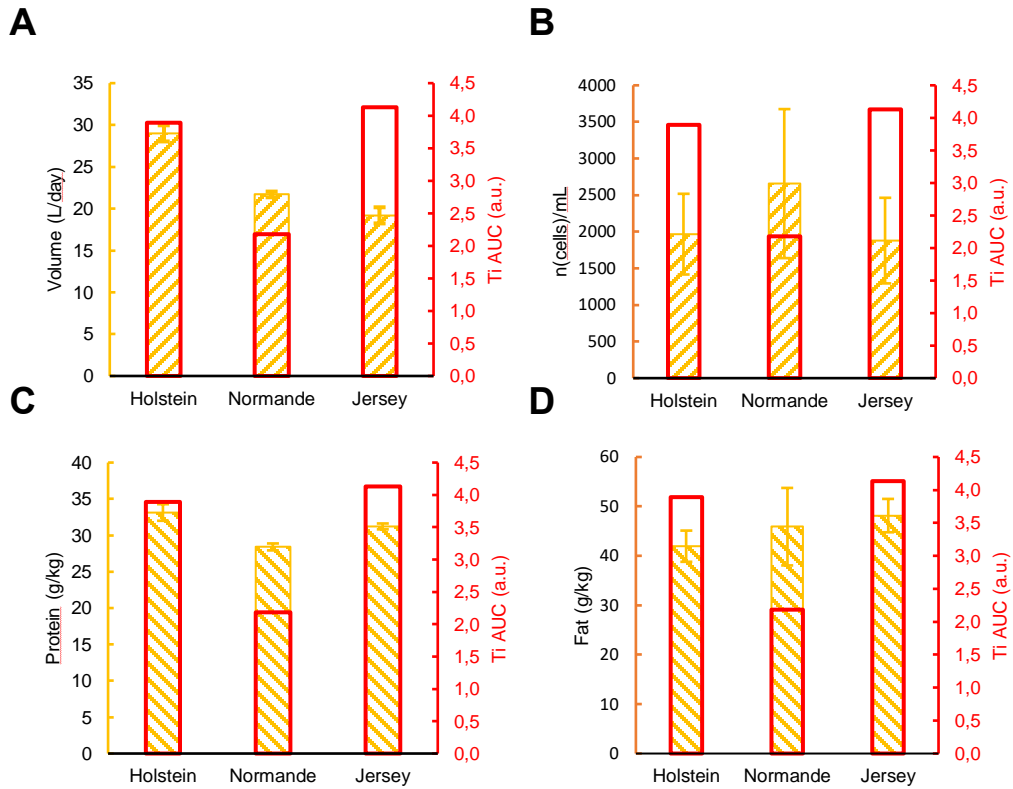
Fig. S6. Comparison of several regions of the same milk pellet. (A). X-ray fluorescence spectra collected at 5.1 keV for different regions of the same pellet IF3, IF5, IF7, and IF4. (B) Elements AUC for two regions of IF4.

A
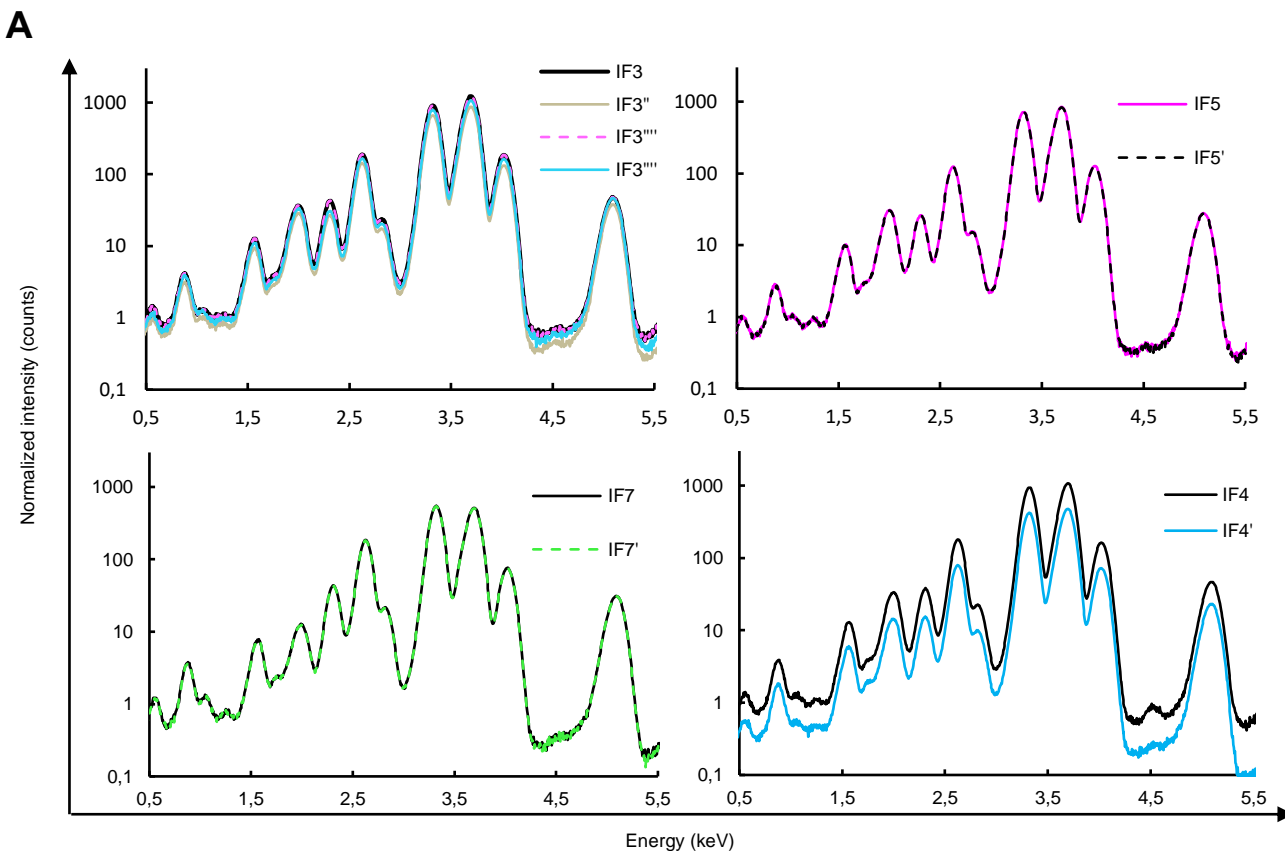
B

	IF4_33470	IF4_33465
O	2,12	4,85
Na	0,99	4,53
Mg	0,92	2,58
Al	13,96	23,42
Si	8,69	8,16
P	256	613
S	367	910
Cl	2322	5288
K	13519	30461
Ca	16022	36209
Ti	1,09	9,48
Cu	3,49	1,92

Fig. S7. Area under curve (AUC) of Ti and Si peaks computed by PyMCA software.

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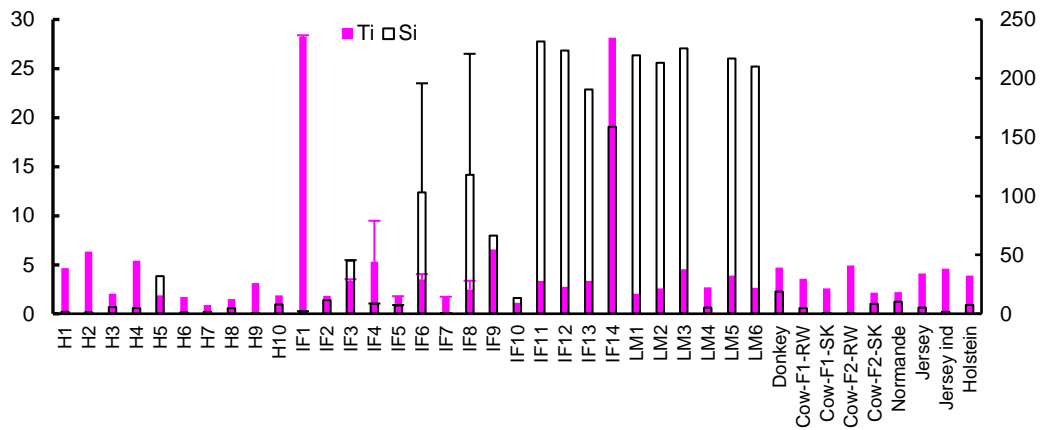
Fig. S8. Workflow for Ti hotspots quantification on µXRF maps (A). Ti map of human H4 region b (H4b) and the average pixel extracted XRF spectra of 3 regions of interest (ROI): a hotspot (ROI1) and regions without hotspot (ROI 2,3). Black arrows point to Ti peaks (B) Ti maps of a region of H2a before ("raw", left) and after Gaussian filtering (right) on which hotspots were detected automatically (in green). White open arrowheads point to hotspots (C) Number of hotspots (particles) detected for each region
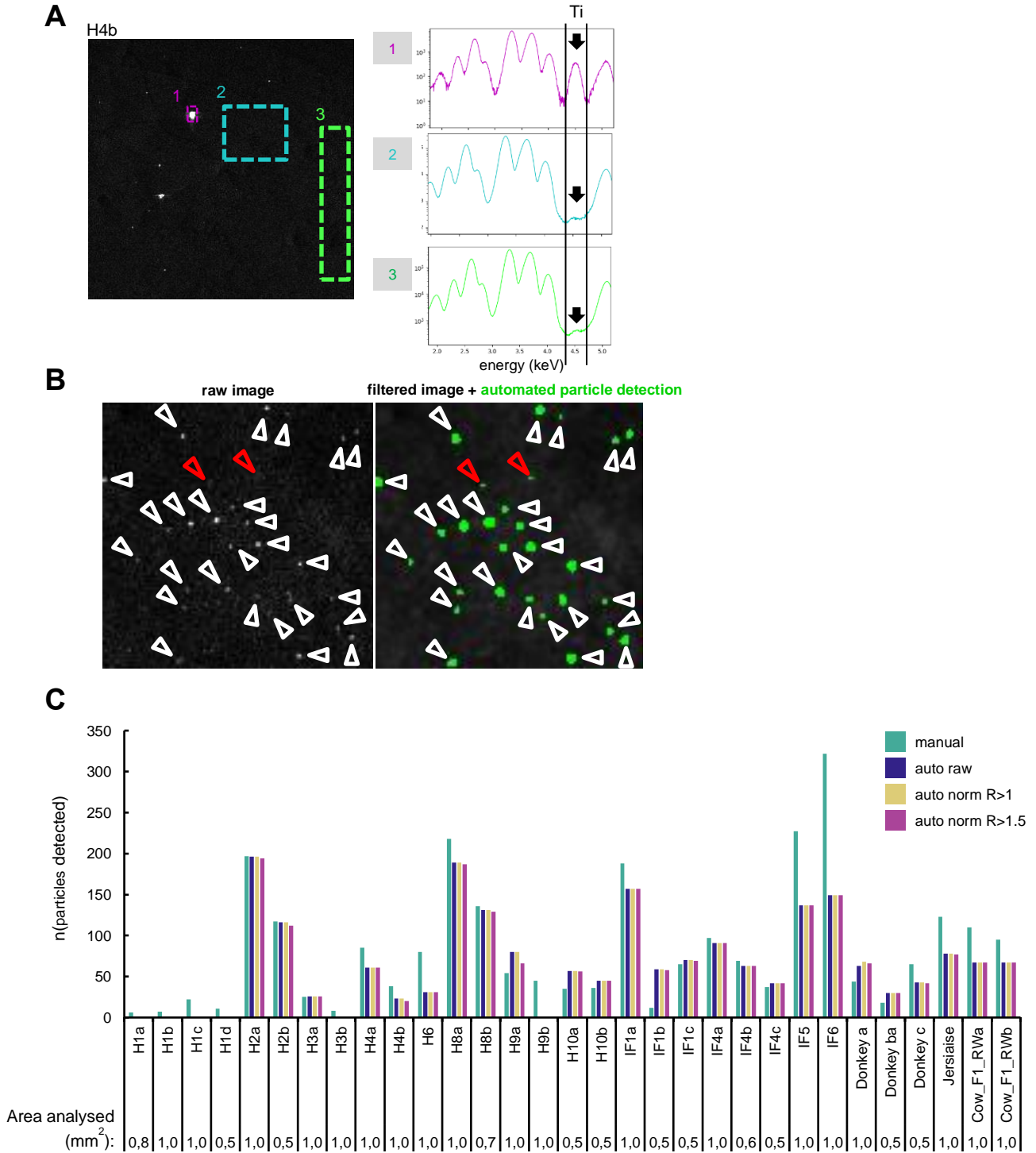
either manually or automatically ("auto", green) without threshold ("auto raw" in blue) or with threshold

of 1 ("R>1", in yellow) or 1.5 ("R>1.5" in pink) signal/noise ratio. Surface area of regions acquired is as indicated.

Fig. S9. Ti maps raw ("raw") or Gaussian filtered ("filtered") with automated detected hotspots (in green). Raw histograms of Ti values (left) with the maximum value chosen for display (green graduation).
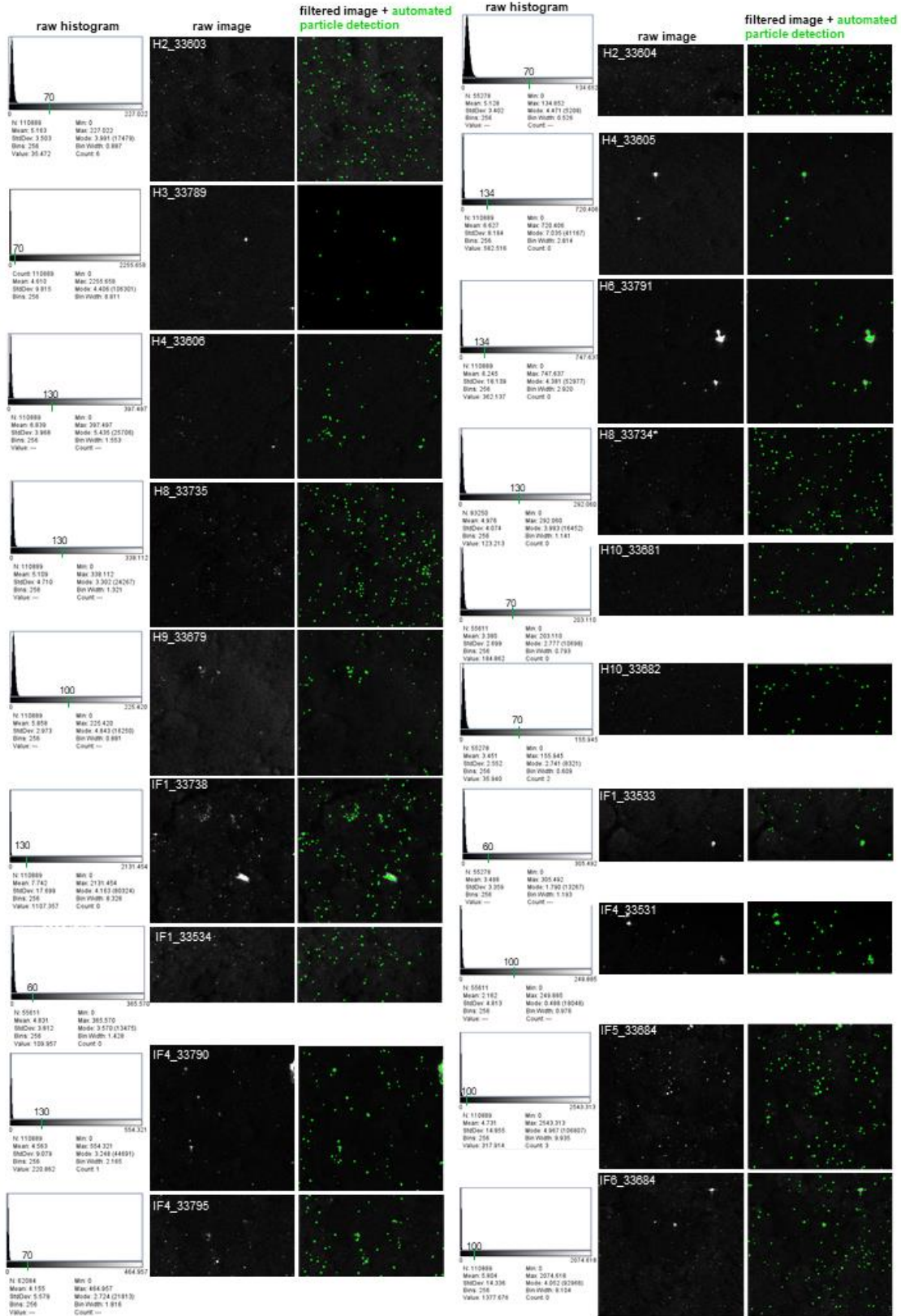
Fig. S10. Ti maps raw ("raw") or Gaussian filtered ("filtered") with automated detected hotspots (in green). Raw histograms of Ti values are shown (left) with the maximum value chosen for display.

raw histogram	raw image	filtered image + automated particle detection

jersey cow_33788

170

0   2074.423
N: 110889   Min: 0
Mean: 9.592   Max: 2074.423
StdDev: 11.240   Mode: 12.155 (53639)
Bins: 256   Bin Width: 8.103
Value: ---   Count: ---

F1_cow_RW_33792

200

0   2700.451
N: 62559   Min: 0
Mean: 7.274   Max: 2700.451
StdDev: 18.527   Mode: 5.274 (53122)
Bins: 256   Bin Width: 10.549
Value: 94.938   Count: 15

F1_cow_RW_33793

130

0   473.267
N: 53784   Min: 0
Mean: 7.701   Max: 473.267
StdDev: 6.769   Mode: 6.470 (10835)
Bins: 256   Bin Width: 1.849
Value: 186.719   Count: 1

donkey_33532*

50

0   440.613
N: 55611   Min: 0
Mean: 3.251   Max: 440.613
StdDev: 4.277   Mode: 2.582 (20377)
Bins: 256   Bin Width: 1.721

donkey_33737

50

0   204.073
N: 55278   Min: 0
Mean: 4.734   Max: 204.073
StdDev: 3.191   Mode: 3.587 (7280)
Bins: 256   Bin Width: 0.797
Value: ---   Count: ---

donkey_33736

70

0   579.130
N: 55944   Min: 0
Mean: 5.338   Max: 579.130
StdDev: 6.414   Mode: 3.393 (18132)
Bins: 256   Bin Width: 2.262
Value: 350.645   Count: 0

Fig. S11. Ti maps raw or Gaussian-filtered with manually detected hotspots (in green). Raw histograms of Ti values is shown (left). Maximal values chosen for display is the maximal value on histogram.

raw histogram	raw image	filtered image + automated particle detection

raw histogram (row 1):
0 — 202.019
N: 110889   Min: 0
Mean: 6.074   Max: 202.019
StdDev: 3.319   Mode: 5.129 (12665)
Bins: 256   Bin Width: 0.789

raw histogram (row 2):
0 — 37.928
N: 88578   Min: 0
Mean: 3.625   Max: 37.928
StdDev: 1.824   Mode: 2.741 (3175)
Bins: 256   Bin Width: 0.148
Value: ---   Count: ---

raw histogram (row 3):
0 — 258.065
N: 110889   Min: 0
Mean: 4.580   Max: 258.065
StdDev: 2.271   Mode: 3.528 (21851)
Bins: 256   Bin Width: 1.008
Value: 211.694   Count: 0

raw histogram (row 4):
0 — 61.359
N: 110889   Min: 0
Mean: 6.183   Max: 61.359
StdDev: 2.457   Mode: 5.633 (4621)
Bins: 256   Bin Width: 0.240
Value: ---   Count: ---

raw histogram (row 5):
0 — 283.976
N: 55278   Min: 0
Mean: 6.034   Max: 283.976
StdDev: 3.104   Mode: 4.992 (10215)
Bins: 256   Bin Width: 1.109
Value: 226.294   Count: 0

raw histogram (row 6):
0 — 73.440
N: 110889   Min: 0
Mean: 4.348   Max: 73.440
StdDev: 2.185   Mode: 3.586 (6278)
Bins: 256   Bin Width: 0.287
Value: 35.286   Count: 0

raw image labels: H9_33680, H1_33518, H1_33530, H1_33588, H1_33602, H3_33794

**filtered image counts:** n=24, n=9, n=7, n=22, n=11, n=8

13

Figure S12. Ti K-edge micro-XANES spectra of human (H1, H2, H4, H9, and H10) breastmilk samples and the corresponding attributions of reference spectra (rutile, anatase, ilmenite and pseudobrookite).
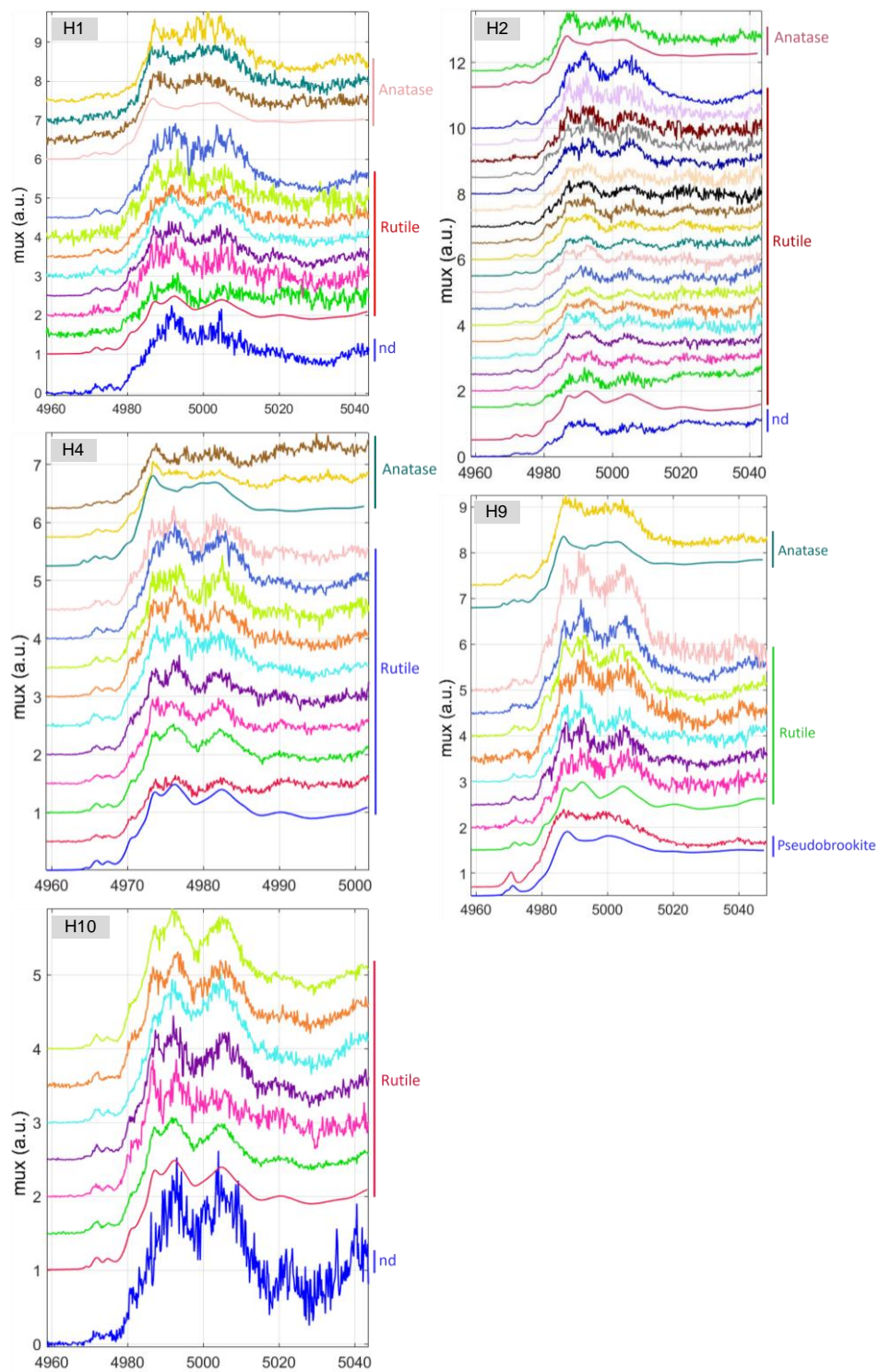
Fig. S13 Ti K-edge micro-XANES spectra of infant formula (IF1, IF4, IF5, and IF6) and animal milk (donkey and Normande (NOR) cow) and the corresponding attributions of reference spectra (rutile, anatase, ilmenite and pseudobrookite).
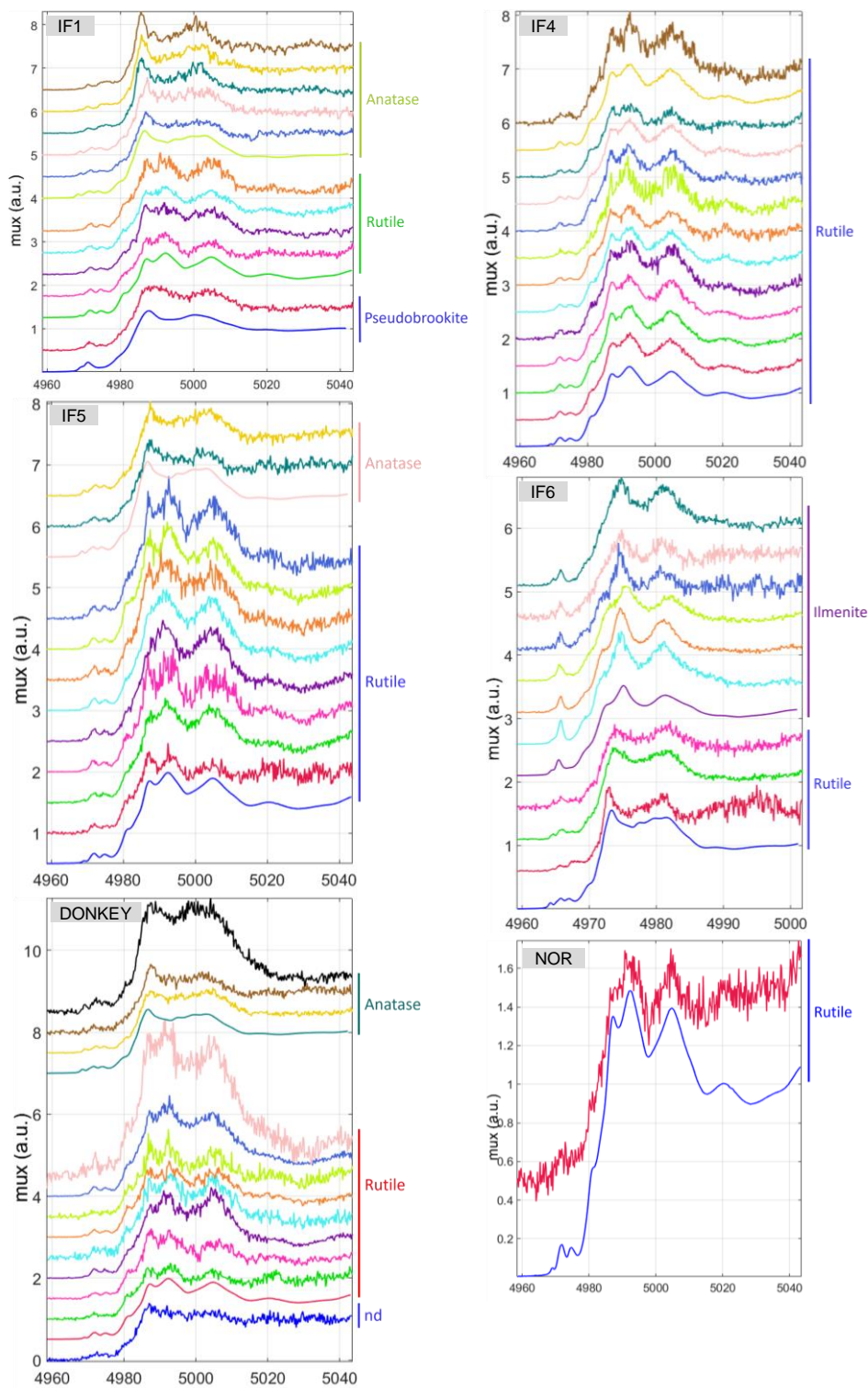
**Fig. S14. Single Particle ICP-MS (SP-ICP-MS) analysis of Ti-containing particles in IF5'
reconstituted milk sample**. (A) The entire set of 1 000 000 measures shows high Ti signal
peaks corresponding to Ti particles ("particles") and a low continued signal corresponding to
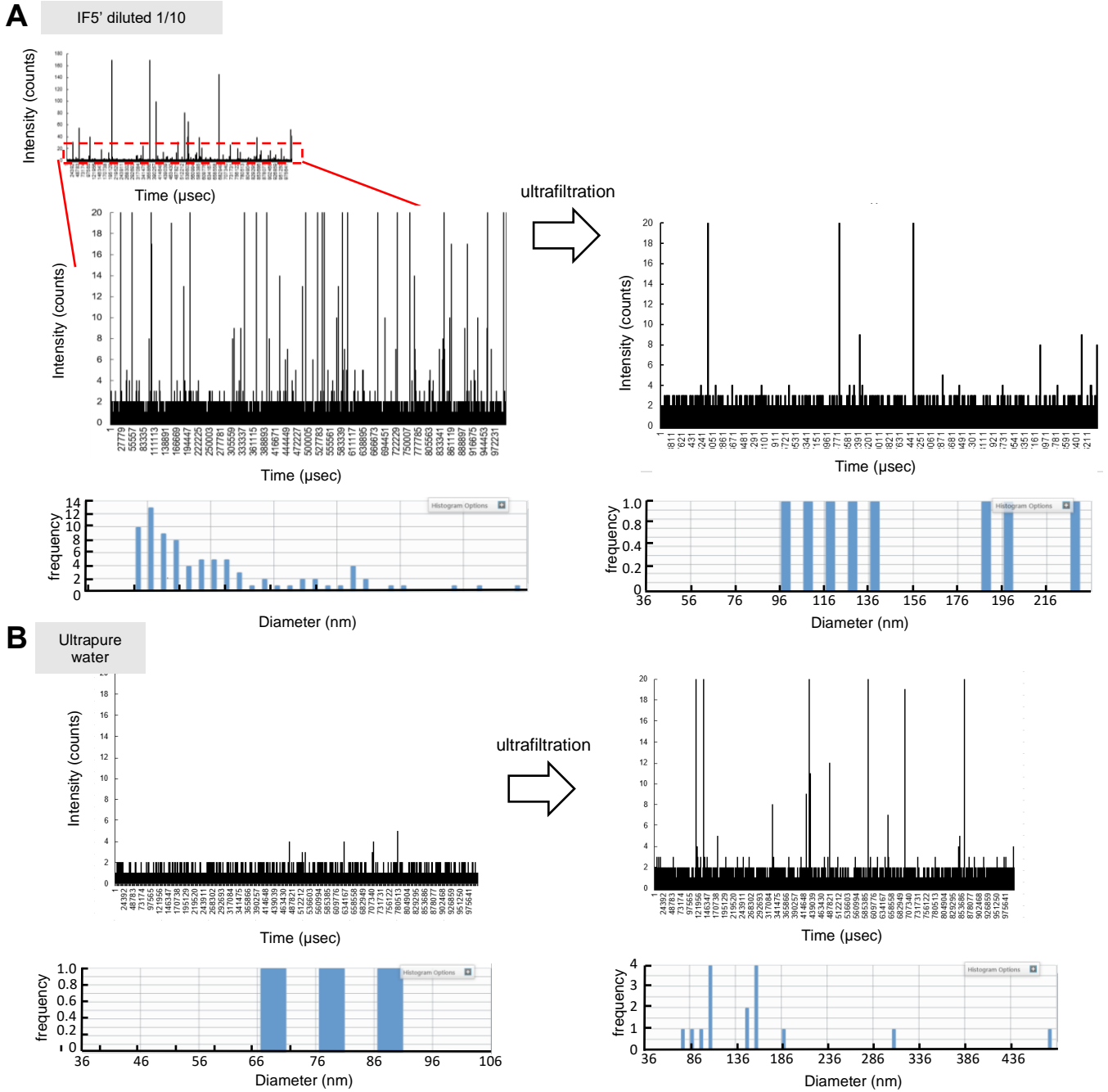soluble Ti ("soluble"). (B) Magnification of the blue dotted box around a single peak.
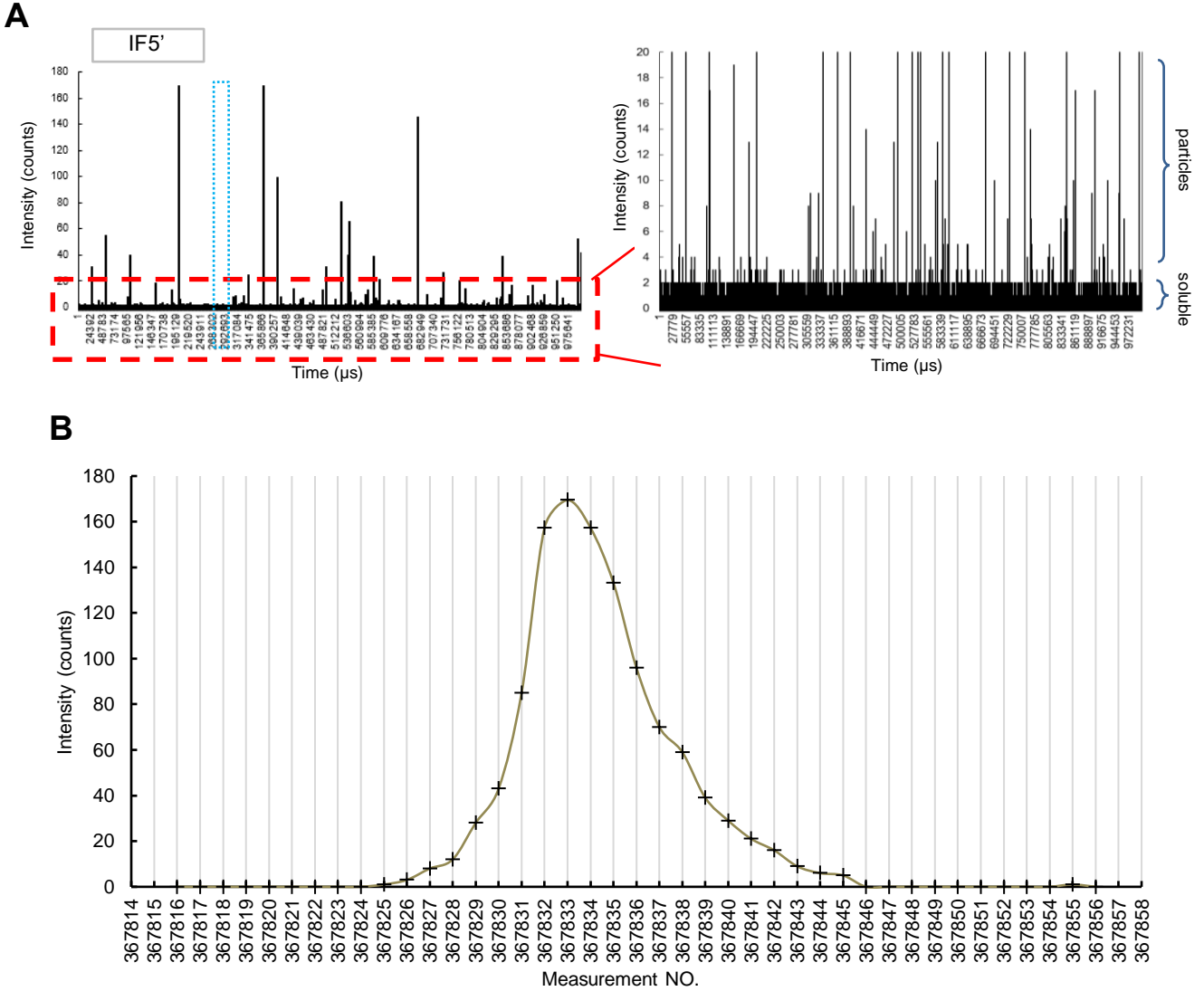
Fig. S15. Size distribution of Ti-containing particles in milk and NIST standard by SP-ICP-MS. Frequency (number of particles) and diameter of particles (in nm) are given.
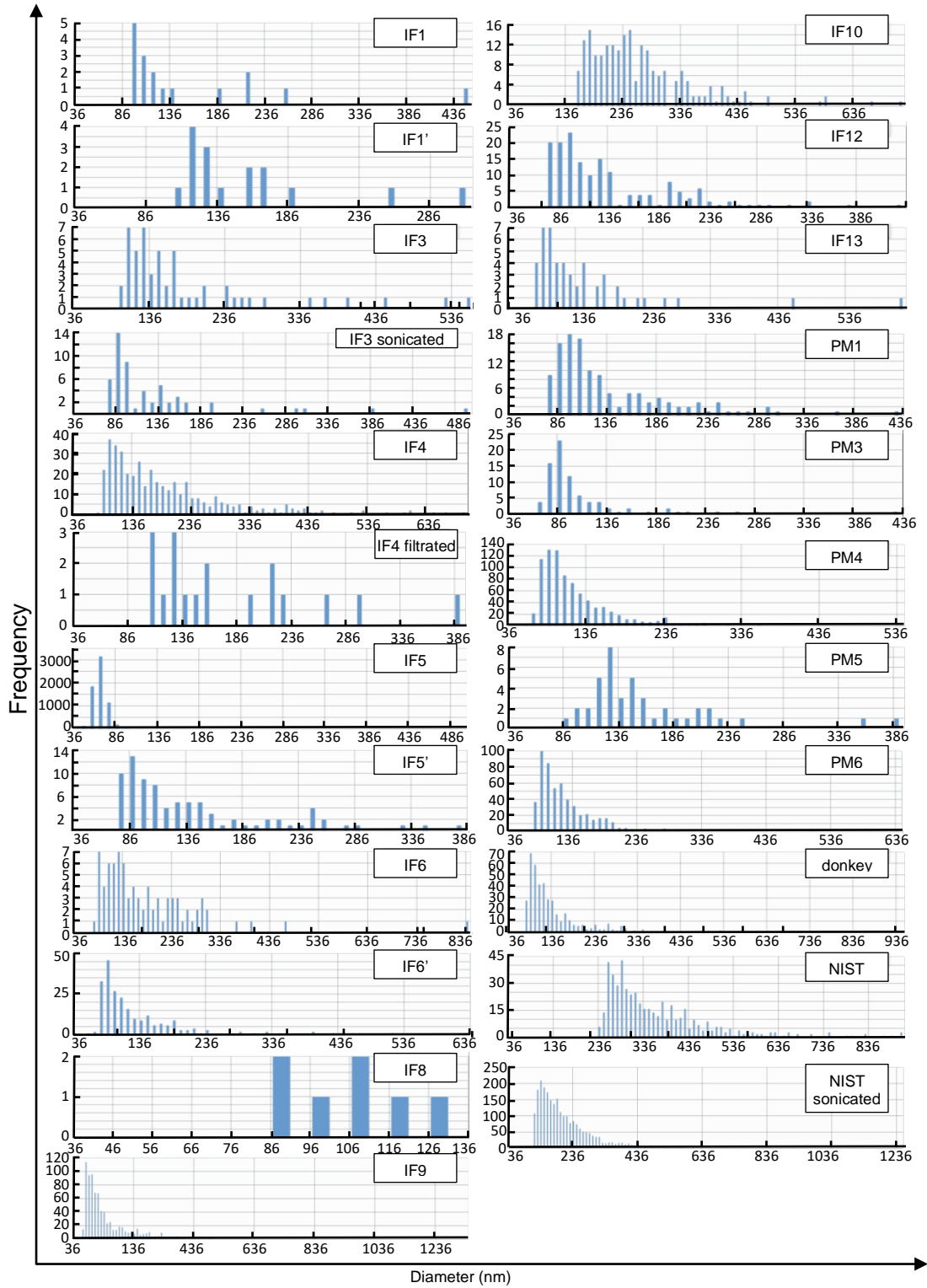
Fig. S16. SP-ICP-MS analysis before and after ultrafiltration of IF5' (A) and ultrapure water (B)

Table S1 Milk samples type, provider, processing and storage

ID	type	provider and origin		initial container	sample processing prior storage	storage condition	storage container
H1 - H10	human raw	Lactarium Port-Royal (AP-HP)	Paris and Paris region (FR)	bottles CE0459 certified	none	frozen	bottles CE0459 certified
IF1-IF14	infant formula	supermarkets and pharmacies	nd	industrial packaging	none	powder	industrial packaging
PM 1-4	cow powder milk	commercial supermarket	France	industrial packaging	none	powder	falcon 50 Corning polypropylene high clarity/polyethylene cap ISO 13485/9001
PM 5	cow powder milk	commercial supermarket	Poland	industrial packaging	none	powder	industrial packaging
PM 6	goat powder milk	commercial supermarket	Austria	industrial packaging	none	powder	industrial packaging
Donkey	donkey raw milk	farm milk	Ariège county (FR)	glass can	none	frozen	falcon 15 Corning polypropylene high clarity/polyethylene cap ISO 13485/9001
Cow-F1-RW	Mix of breeds, raw tank milk	Farm 1	Paris region (FR)	Plastic bottle	none	frozen	"
Cow-F1-SK	Mix of breeds, skimmed tank milk	Farm 1	Paris region (FR)	Plastic bottle	skimmed	frozen	"
Cow-F2-RW	Mix of breeds, raw tank milk	Farm 2	Paris region (FR)	Plastic bottle	none	frozen	"
Cow-F2-SK	Mix of breeds, skimmed tank milk	Farm 2	Paris region (FR)	Plastic bottle	skimmed	frozen	"
Jersey	Blended milk of 6 Jersey cows collected on day 18/03/2021	INRAE UEP	Normandie region (FR)	pyrex glass bottle	none	frozen	falcon 50 Corning polypropylene high clarity/polyethylene cap ISO 13485/9001
Jersey ind	raw milk from individual cow collected on day 18/03/2021	INRAE UEP	Normandie region (FR)	pyrex glass bottle	none	frozen	"
Holstein	Blended milk from 6 Holstein cow collected on day 18/03/2021	INRAE UEP	Normandie region (FR)	pyrex glass bottle	none	frozen	"
Normande	Blended miilk of 6 Normande cows collected on day 18/03/2021	INRAE UEP	Normandie region (FR)	pyrex glass bottle	none	frozen	"

Table S2 Additional information on PM and IF samples

ID	type	Information provided by manufacturer	Additional information
PM1	cow powder milk	half skimmed	
PM2	cow powder milk	half skimmed	
PM3	cow powder milk, organic	half skimmed organic	
PM4	cow powder milk	raw	
PM5	cow powder milk	raw	
PM6	goat powder milk	raw	
IF1	infant formula	0-6 months old thickened	
IF2	infant formula	6-12 months old organic	
IF3	infant formula	0-6 months old	batch 09/2022
IF4	infant formula	0-6 months old	IF3 newer batch 06/2023
IF5	infant formula	6-12 months old thickened	
IF6	infant formula	6-12 months old	
IF7	infant formula	12-36 months old	
IF8	infant formula	12-36 months old	
IF9	infant formula	0-6 months old thickened	
IF10	infant formula	0-6 months old organic	
IF11	infant formula	12-36 months old organic	
IF12	infant formula	0-6 months old organic	
IF13	infant formula	0-6 months old organic	
IF14	infant formula	0-6 months old thickened	
IF1'	infant formula	0-6 months old	IF1 new batch 12/2023
IF5'	infant formula	6-12 months old	IF5 new batch 12/2023
IF6'	infant formula	6-12 months old	IF6 new batch 12/2023

Table S3. Size and concentration of Ti-containing particles in milk, % of soluble Ti and % of particles as detected by SP-ICP-MS. Concentration is given as Ti particles per liter of milk based on the manufacturer's recommendations to reconstitute powder milk for infant (IF) or adult (PM) consumption.

ID	Most frequent size(nm)	Mean size (nm)	Median size (nm)	% <100nm	Ti part/L	% soluble	% particles
Water	86	76	76	100	860 000		
Water filtrated	116	160	146	19	4 610 000		
IF1	96	154	116	29	10 290 000	97	3
IF1'	116	155	131	0	9 681 000	96	4
IF3	126	185	146	4	32 067 000	61	39
IF3 sonicated	86	132	96	53	33 285 000	75	25
IF4	96	191	161	15	246 855 000	19	81
IF4 filtrated	156	178	151	0	10 899 000	93	7
IF5	46	66	66	99	3 996 909 000	87	13
IF5'	96	141	116	38	50 820 000	76	24
IF5' filtrated	116	149	131	13	4 830 000	99	1
IF5' sonicated	66	134	106	42	125 853 000	52	48
IF6	126	184	156	14	50 211 000	86	14
IF6'	56	133	106	46	143 997 000	84	16
IF8	96	103	106	43	4 830 000	99	1
IF9	216	265	246	0	113 631 000	26	74
IF10	46	148	116	40	429 030 000	28	72
IF12	96	138	116	39	98 616 000	79	21
IF13	86	148	116	31	29 652 000	85	15
PM1	96	138	116	34	50 414 000	79	21
PM3	56	110	86	66	33 474 000	89	11
PM4	46	122	106	46	348 096 000	76	24
PM5	126	158	141	7	16 940 000	95	5
PM6	56	126	106	41	220 640 000	42	58
Donkey	96	143	116	36	79 971 920	42	58
NIST 10µg/L	296	365	326	0	146 650 000	3	97
NIST sonicated 10µg/L	126	214	186	0	705 310 000	10	90