

Supplementary Information

Establishment of region-, age- and gender-specific reference intervals for aldosterone and renin in China using a novel sandwich chemiluminescent immunoassay

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Table S1. Observed results for LoQ verification. Results outside the allowable TE window are shown in bold.

	ALD (ng/L)		Renin (mU/L)	
	Sample 1	Sample 2	Sample 1	Sample 2
Target Value	33.6	37.8	1.22	1.02
Error Window				
Lower Limit	28.55	32.10	1.04	0.87
Upper Limit	38.63	43.44	1.40	1.17
Test Day				
1	33	38	1.21	1.08
2	33.7	37.4	1.26	1.12
3	33.1	38	1.24	1.05
4	33.2	38.2	1.13	1.07
5	33	37.8	1.17	1
6	33.1	38.1	1.25	1.04
1	33	37.9	1.3	1.04
2	32.7	38.4	1.27	0.913
3	33.1	38.4	1.17	1
4	33.5	38	1.24	0.938
5	33.6	37.8	1.21	1
6	33.7	37.1	1.15	1.01
1	34.6	37.9	1.18	0.993
2	34.6	37.8	1.26	1.05
3	34.7	38.1	1.25	0.975
4	33.8	36.8	1.26	0.969
5	34.3	36.8	1.19	1.03
6	33.9	37.4	1.29	1
CV	1.86%	1.28%	4.06%	4.97%
Outliers	0	0	0	0

Assuming 5% Type I and Type 2 error risks ($\alpha=\beta=0.05$). Upper and lower allowable total error windows were calculated for each sample as the target value $\pm 15\%$.

Table S2. Within-run, between-run and total imprecision of aldosterone.

Sample	Mean (ng/L) (N=25)	Within-Run		Between-Day		Total	
		SD (ng/L)	%CV	SD (ng/L)	%CV	SD (ng/L)	%CV
P1	1748	36.9	2.11	12.7	0.73	39.0	2.23
P2	814	14.6	1.79	0	0	14.6	1.79
P3	407	4.74	1.17	3.29	0.81	5.77	1.42

P4	210	3.35	1.59	1.75	0.83	3.78	1.80
P5	102	1.42	1.39	4.09	4.02	4.33	4.25
P6	41.0	1.03	2.52	1.73	4.22	2.01	4.91
QC1	59.1	1.09	1.84	2.80	4.74	3.01	5.09
QC2	338	7.77	2.30	12.7	3.75	14.9	4.40

Table S3. Within-run, between-run and total imprecision of renin.

Sample	Mean (mU/L) (N=25)	Within-Run		Between-Day		Total	
		SD (mU/L)	%CV	SD (mU/L)	%CV	SD (mU/L)	%CV
P1	881	8.67	0.98	3.78	0.43	9.46	1.07
P2	420	5.49	1.31	0	0	5.49	1.31
P3	213	5.25	2.47	2.08	0.97	5.65	2.65
P4	112	2.34	2.09	1.09	0.97	2.58	2.30
P5	41.2	0.59	1.42	0.57	1.39	0.82	1.98
P6	15.9	0.65	4.11	0	0	0.65	4.11
QC1	37.2	0.87	2.35	0.82	2.20	1.20	3.22
QC2	101	1.38	1.37	0	0	1.38	1.37

Table S4. Linearity of aldosterone.

	0/4	1/4	2/4	3/4	4/4
Mean (ng/L)	31.7	491	894	1427	1869
Expected concentration (ng/L)	31.7	491	950	1409	1869
Recovery	100.0%	100.0%	94.1%	101.2%	100.0%

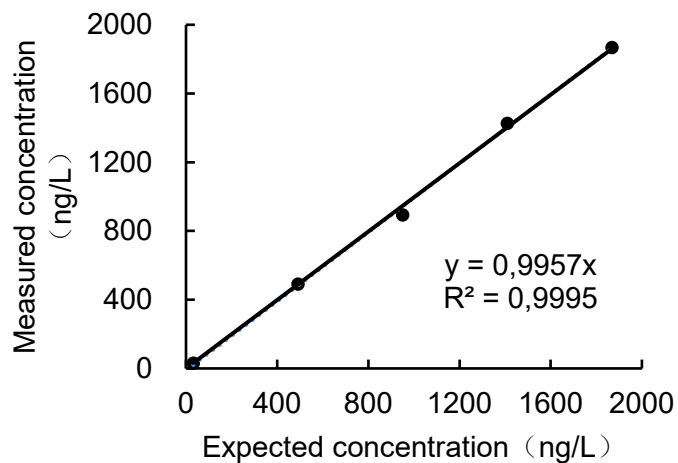
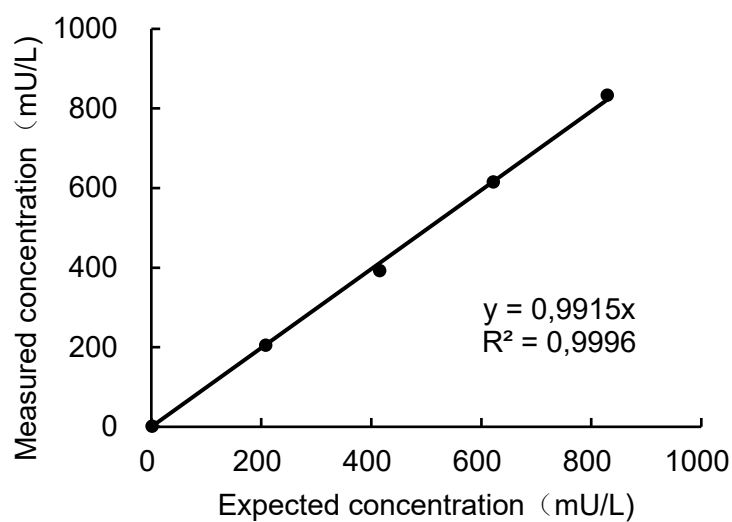


Figure S1. Linearity of aldosterone.

Table S5. Linearity of renin.

	0/4	1/4	2/4	3/4	4/4
Mean (mU/L)	1.09	205	392	615	833
Expected concentration (mU/L)	1.06	208	415	622	829
Recovery	102.5%	98.4%	94.5%	98.9%	100.5%

**Figure S2.** Linearity of renin.**Table S6.** Trueness of aldosterone.

	GBW09285 (ng/L)	GBW09284 (ng/L)	GBW09283 (ng/L)
1	515	213	88.8
2	519	216	89.3
3	507	210	86.9
4	506	210	91.5
5	512	216	87.1
6	507	199	91.8
Mean	511	210.7	89.2
Target value	526	201	82.7
Bias	-2.91%	4.75%	7.94%

Table S7. Recovery of aldosterone.

	Sample A (ng/L)	Sample B (ng/L)	Concentration After Mixed (ng/L)	Measured Concentration (ng/L)	Recovery
Sample 1	295	54.6	82.5	76.5	92.7%
Sample 2	295	54.6	77.0	72.4	94.1%
Sample 3	295	54.6	66.7	66.6	99.9%
Sample 4	472	43.1	258	247	95.9%
Sample 5	472	43.1	150	143	95.3%
Sample 6	472	43.1	86.0	80.9	94.0%
Sample 7	1700	38.4	205	203	98.9%
Sample 8	1700	38.4	172	168	97.8%
Sample 9	1700	38.4	123	119	96.7%

Table S8. Recovery of renin.

	Sample A (mU/L)	Sample B (mU/L)	Concentration After Mixed (mU/L)	Measured Concentration (mU/L)	Recovery
Sample 1	444	14	229	223	97.4%
Sample 2	444	14	122	115	94.4%
Sample 3	444	14	57.0	52.2	91.5%
Sample 4	585	17.6	73.9	75.9	102.7%

Table S9. The dilution of aldosterone.

Dilution	Expected concentration (ng/L)	Measured concentration 1 (ng/L)	Measured concentration 2 (ng/L)	Measured concentration 3 (ng/L)	Mean (ng/L)	Recovery
0	1868	1829	1906	1959	1868	
2×	934	931	862	860	897	96.0%
5×	374	365	376	366	371	99.2%
10×	187	179	182	180	181	96.7%
20×	93.4	89.6	89.3	90.8	89.5	95.8%
50×	37.4	37.1	37.7	38.3	37.4	100%

Table S10. The dilution of renin.

Dilution	Expected concentration (mU/L)	Measured concentration 1 (mU/L)	Measured concentration 2 (mU/L)	Measured concentration 3 (mU/L)	Mean (mU/L)	Recovery
0	962	958	966	960	962	
2×	481	473	455	464	464	96.5%
5×	192	181	179	182	180	93.6%
10×	96.2	93.4	83.3	91.7	88.4	91.8%

Table S11. Interference of aldosterone.

Interferent	Spiked amount	ALD concentration (ng/L)	Interference
Hemoglobin	600 mg/dL	95.5	-2.55%
		354	4.73%
Triglycerides	2000 mg/dL	97.4	-0.61%
		339	0.30%
Bilirubin	40 mg/dL	91	-7.99%
		322	-4.17%
Human anti-mouse antibody	40 ng/mL	97.5	-0.51%
		332	-1.78%
Antinuclear antibodies	310 AU/mL	97.2	-0.82%
		334	-1.18%
Rheumatoid factor	1400 IU/mL	98.2	0.20%
		329	-2.66%
Biotin	0.5 mg/dL	95.4	-1.65%
		327	1.24%
K ₂ -EDTA anticoagulation	22.75 μ mol/mL	96.9	2.76%
		333	2.15%
Glucose	10 mg/mL	95.8	-2.24%
		333	-1.48%
Ascorbic acid	6 mg/dL	96.7	-1.33%
		334	-1.18%
Acetaminophen	20 mg/dL	96.9	-1.12%
		327	-3.25%
Acetylsalicylic acid	65.2mg/dL	96.6	-1.43%
		338	0.00%

Table S12. Cross-reactivity of aldosterone.

Cross-reactants	Spiked amount	ALD concentration (ng/L)	Cross-reactivity
Eplerenone	1.99 μ g/dL	94.6	-2.47%
		336	4.02%
Dehydroepiandrosterone	100000 ng/mL	96.3	2.01%
		339	-2.31%
Estradiol	100000 ng/mL	95.5	4.49%
		335	0.30%
Estriol	10000 ng/mL	90.4	-3.42%
		330	3.13%
17-alpha-hydroxyprogesterone	100000 ng/mL	95.9	-0.42%
		320	-2.44%

Spironolactone	100000 ng/mL	94.1	-1.88%
		337	-2.60%
Progesterone	100000 ng/mL	97.3	0.93%
		327	2.83%
Testosterone	100000 ng/mL	94.4	0.85%
		315	-6.53%
Estrone	10000 ng/mL	90.9	-6.29%
		318	-1.55%
Dexamethasone	200000 ng/mL	95.7	1.38%
		334	-3.75%

Table S13. Interference of renin.

Interferent	Spiked amount	Renin concentration (mU/L)	Interference
Hemoglobin	500 mg/dL	13.5	6.30%
		42.6	6.77%
Triglycerides	3000 mg/dL	12.7	0.00%
		38.1	-4.51%
Bilirubin	20 mg/dL	13.9	3.73%
		39.7	7.88%
Beta ₂ -microglobulin	50 µg/mL	19	-4.52%
		38.2	-4.26%
Angiotension I	10 ng/mL	14.3	6.72%
		37.1	-1.07%
Angiotension II	1.0 ng/mL	13.4	0.00%
		37.7	0.53%

Table S14. 24-h urinary sodium of the five regions.

24-h UNa (mmol/24h)	Henan	Gansu	Beijing	Jiangsu	Guangdong
Yin et al. 2023 ^a	221		102		
Tan et al. 2019 ^b	244	200 ^c	200	220	136
Hipgrave et al. 2016 ^b	205	200 ^c	200	168	126
Wang et al. 2022 ^b			137		
Li et al. 2021 ^b			178		
Sun et al. 2020 ^b	190	180	160		110
Guo et al. 2019 ^b				178	

^aData were described as median, ^bdata were presented as mean, ^c represents the substitution of Shaanxi Province for Gansu Province, as Shaanxi is adjacent to Gansu and has similar dietary habits.

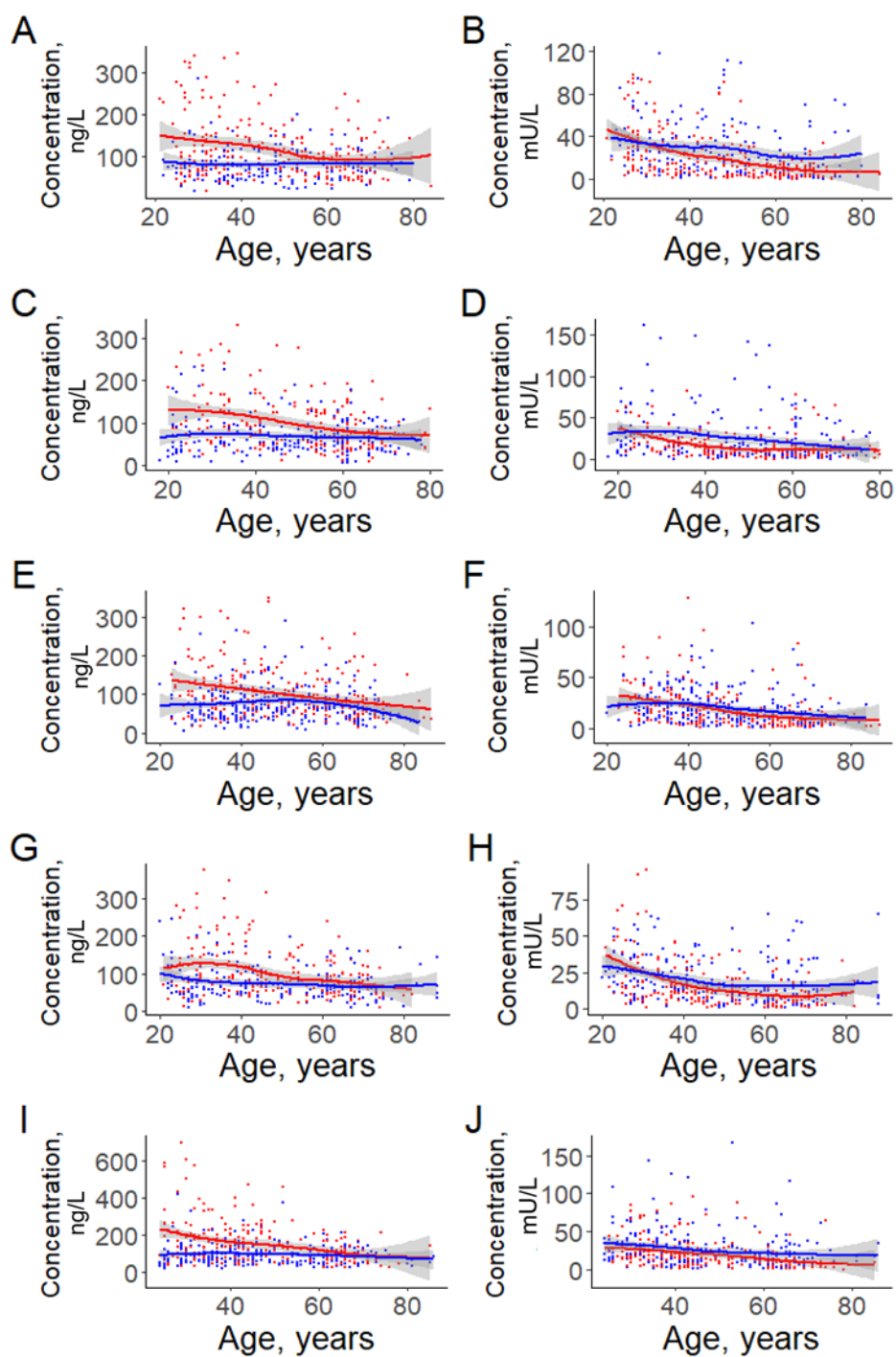


Figure S3. Age- and sex-specific reference value scatterplots for: (A) ALD of Henan; (B) Renin of Henan; (C) ALD of Gansu; (D) Renin of Gansu; (E) ALD of Beijing; (F) Renin of Beijing; (G) ALD of Jiangsu; (H) Renin of Jiangsu; (I) ALD of Guangdong; (J) Renin of Guangdong. Red dots represent females; blue dots represent males.

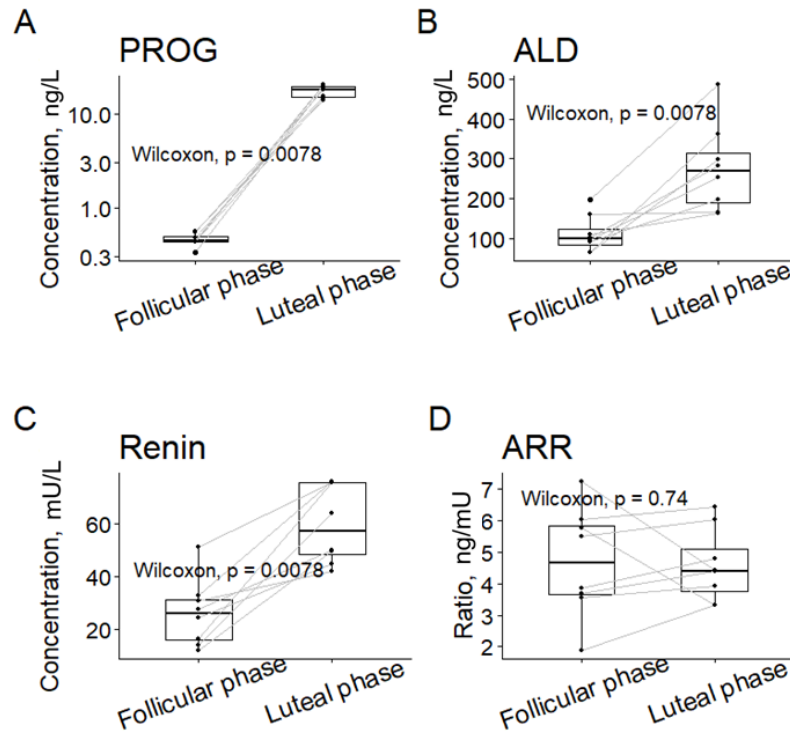


Figure S4. Menstrual cycle-specific boxplots for: (A) PROG (progesterone); (B) ALD; (C) Renin; (D) ARR.

References

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