## A systematic review of methods to measure menstrual blood loss

## SUPPLEMENTAL TABLE 4

## Further validation of methods.

Author and year (reference)	Method	Study population	N (n) <sup>a</sup>	Recovery of blood/iron	Internal consistency	Other validation
AH						
Magnay et al., 2011 [1]	Modified AH method (rapid AH technique for SAP-c products)	Simulated menstrual fluid	N/A (63 towels with simulated fluid samples; 21 for each type)	Linearity was confirmed, and $\geq 85\%$ blood recovery was reproducibly achieved from 0.05–30 mL at 5–100% simulated menstrual fluid compositions (except at low volume/high dilution equivalent to <4 mL)	Intra-assay coefficient of variation was <4.5% for both 5 and 20 mL volumes of blood applied to normal towels (n = 20)	Linear regression analysis of reference versus test method gave a coefficient of determination ( $r^2$ ) of 0.991 ( $P$ <.0001, n = 63)
				Mean extraction efficiency (with absorbency conversion factors): normal, 91.3%; long, 94.3%; night, 94.5%		
				There was no significant difference in hemoglobin recovery between towel types (one-way ANOVA, P>.05)		
Magnay et al. 2010 [2]	Modified AH (SAP version)	Simulated menstrual fluid	N/A (6/10 simulated fluid samples for each experiment)	90% recovery was reproducibly achieved for up to 30 mL of applied volume (n = 10/11 per volume) and at all tested simulated menstrual	Precision improved as the applied volume increased from 5 mL to 20/30 mL (10 replicates)	_
				fluid compositions (n = 6 per composition), except at low volume or high dilution equivalent to $<2$ mL	Coefficients of variation (10 replicates): normal, 10.6–6.3; long, 13.4– 5.6; night, 9.4–6.0	
Hurskainen et al., 1998 [3]	Modified AH method	N/A	N/A (blood)	Mean blood recovery of 10– 20 mL from tampons and 10–	_	_

Author and year (reference)	Method	Study population	N (n) <sup>a</sup>	Recovery of blood/iron	Internal consistency	Other validation
	(Stomacher <sup>®</sup> blender and absorbance at A 564; menstrual diary)			200 mL from towels (n = 3 per volume): 93%		
Gannon et al., 1996 [4]	Modified AH method (detergent extraction; photometric analysis; calibration factor)	Women with heavy MBL	146 (292) (time-expired whole blood)	There was a linear relationship between 10 and 250 mL of blood (gradient, 1.0; n = 3 per volume)	There was good intra- individual correlation between two repeat measurements (r = 0.713, P <.001)	_
Vasilenko et al., 1988 [5]	Modified AH method (volumetric test; non-caustic chemicals; standard curves)	Women with normal MBL and those diagnosed with dy sfunctional uterine bleeding	10 (35) (whole blood solution; hematocrit 50%)	There was a significant linear relationship between 0 and 10 mL of blood ( <i>P</i> <.0001) for the four products; on average, extraction efficiency was 96.9% Thin maxi pads had a significantly lower slope than blood alone ( <i>P</i> <.001); extraction efficiency: 7.5 mL, 85.2%; 10.0 mL, 74.8% Extraction efficiencies of both brands of tampons were 99.0%	The variation in individuals from menstrual period to period (1.5 SD units) was as great as the variation between women (1.5 SD units); thus, it is advisable to determine blood losses from more than one cycle per woman	_
van Eijkeren et al., 1986 [6]	Modified AH method (Stomacher blender and modified equation for calculation of MBL)	Women with self-perceived heavy MBL	21 (21) (outdated whole blood)	Mean recovery of blood from tampons and towels (using equation that subtracts blanks and incorporates post- filtration volume; n = 3-4 per volume): 10 mL, 98% 40 mL, 102% 80 mL, 98% 140 mL, 99% 200 mL, 98%	The coefficient of variation using this technique varied between 6.1% and 7.5% The intra-assay variability (variation on one day) was 3.3%; the inter-assay variability (variation over several days) was 3.8% Variation between observers was negligible	_

Author and year (reference)	Method	S tudy population	<b>N</b> ( <b>n</b> ) <sup>a</sup>	Recovery of blood/iron	Internal consistency	Other validation
Desai et al., 1982 [7]	Modified AH method (Stomacher blender; automation)	N/A	N/A (blood)	Both types of pads, "gave close to 100% recovery", using 500 mL of sodium hydroxide and 15 min of extraction per pad	Intra-individual and intracycle variation was low	External validation (Indian population)
Newton et al., 1977 [8]	Modified AH method (Stomacher blender; automation)	N/A	N/A (peripheral blood)	Mean recovery of blood (5– 200 mL; $n = 4$ per volume) from seven tampons and pads was 82–93% (coefficient of variation on replicate analysis was 3.4–11.0%) Mean recovery of 20 mL of blood added to each of 13 products ( $n = 4$ ): 20.3 mL (range, 18.4–22.3 mL)	_	_
Shaw et al., 1972 [9]	Modified AH method (increased incubation time)	N/A	N/A (outdated whole blood)	Recovery of blood (n = 3): Pads: 0.1 mL, 95–103% 0.5 mL, 98–101% Pads and tampons: 1.0 mL, 95–100% 2.0 mL, 95–100% 3.0 mL, 100–104% 4.0 mL, 96–105% 5.0 mL, 96–100% Average recovery of vaginal MBL from tampons after	_	_
				insertion for 6–16 h: 102.5% (range, 100–107%; n = 6)		
Hallberg & Nilsson, 1964 [10]	АН	Healthy women tested the accuracy of the method	2 (24) (simulated fluid samples)	Recovery of dried blood (n = 10 per test): After 5 h, 87.4% (SE, 1.0) After 20 h, 96.3% (SE, 0.5)	Consistent losses were seen over 12 cycles in two women	_
MFL						
Gudmundsdottir et al., 2009 [11]	MFL	Women with diagnosed heavy MBL or normally menstruating	78 (78)	_	_	External validation (adults vs teenagers; users of oral contraceptives vs non-users)

Author and year (reference)	Method	Study population	<b>N</b> ( <b>n</b> ) <sup>a</sup>	Recovery of blood/iron	Internal consistency	Other validation
		women/adolescents				
Fraser et al., 2001 [12]	Regression estimation of MBL from total MFL	Women with self-perceived normal MBL, heavy MBL, or previous heavy MBL	24 (48)	-	Median inter-cycle variability: MBL, 18% MFL, 26%	-
Measurement of iron	labelled blood					
Cheyne & Shepherd, 1970 [13]	Chemical [14] and atomic absorption spectrophoto- metry analysis of iron recovery	Menstruatingwomen	N/A (25 used pads)	Recovery of added iron (n = 3-4): atomic absorption, 97.5–105% chemical determination, 98.1– 100.9%	SD of difference in duplicate estimations among six washes: either method, 0.1 mg Fe/100 mL Average SD from mean, ±3%	_
					SD of differences in samples (mg Fe/100 mL): atomic absorption, 0.029 chemical, 0.067 Average SD from mean (%): atomic absorption, ±0.90 chemical, ±2.24	
Tauxe, 1962 [15]	Radioactivity counting dome	N/A	N/A	_	Experimental accuracy of counting dome from series of six different quantities of venous blood, 2 SDs = 1.72 (1.6% of mean 104.89 mL) 0.8% error of radioactivity over 10 different sites on the	_
Baldwin et al., 1961	Fe <sup>59</sup> radioactive	N/A	N/A		dome The error in recovery of blood	
[16]	iron	N/A	(pooled blood)	_	samples was consistently less than 0.5 mL per 20 mL	_
PBAC						
MacEachern et al., 2015 [17]	e-PBAC e-BQ	Women without menstrual disorder	47 (47)	_	_	External validation (oral contraceptive users vs non-users)
Goshtasebi et al., 2015 [18]	PBAC SF-36	Women with self-perceived HMB	76 (304)	-		External validation (Iranian women)
Hald & Lieng, 2014 [19]	Modified PBAC (revised icons)	Untreated women with self- perceived light, normal, or	118 (236)	-	Intra-class correlation coefficient for PBAC	_

				Recovery of blood/iron	Internal consistency	Other validation
		heavy MBL reporting on two cycles			measurements in two cycles, 0.86 Inter-individual variation in PBAC scores was high	
2012 [20] bleedin		Adolescent girls	66 (66)	-	_	External validation (adolescents)
Sanchez et al., 2012 PBAC [21]	2	Menstruating adolescents	73 (73)	-	-	External validation (adolescents)
[22] (revise		Women with or without menstrual disorder	160 (320)	_	_	External validation (Iranian women)
Biri et al., 2007 [23] PBAC	C [24]	Menstruating women	600 (600)	-	-	External validation (PBAC in Turkish women)
Shapley & Redman, PBAC 1995 [25]	2	Women aged $\geq 40$ years	283 (283)	-	-	PBAC may overestimate MBL in the general community
Janssen et al., 1995 Modif [26]		Women with self-perceived normal or heavy MBL or with unexplained anemia	288 (489)	_	85% of participants were consistent in assessment of their second menses	Agreement between participant and investigator scores was good
Higham et al., 1990 PBAC [27]		Women with a range of MBLs	28 (55)	-	_	Agreement between gynecologist and participant scores was good (within 2 SDs)
Menstrual pictogram						,
version	gram (SAP	Women with self-perceived light, normal, or heavy MBL	119 (235)	_	_	Participant and expert ratings concurred for 59% (1,957/3,315) of towels ( $\kappa = 0.46$ ); in 95% of cases the scoring discrepancy was less than or equal to one icon category
Larsen et al., 2013 Modif [29] menst: pictog (exclut extran- MBL)	rual gram ding eous	Women with confirmed HMB	170 (169)	_	_	External validation (North American treated population)
	,	Premenopausal women with	12	There was a significant	There was a high level of	There was no significant

Author and year (reference)	Method	Study population	N (n) <sup>a</sup>	<b>Recovery of blood/iron</b>	Internal consistency	Other validation
[30]	pictogram (SAP version)	regular menstrual cycles who had not previously used a graphical method to assess menstrual loss	(N/A) (simulated menstrual fluid)	correlation between the volume of blood applied and that recovered by AH $(r^2 = 0.992, P < .0001)$ , and between the volume of fluid applied and the soiled towel weight $(r^2 = 0.998, P < .0001)$	agreement between individual scores: for 112 (83%) of 135 towels, the participants' readings either completely concurred or differed by only a single icon; there was no significant difference between the 12 sets of scores (ANOVA on ranks, <i>P</i> >.05)	difference between expert and participant ratings (ANOVA on ranks, <i>P</i> >.05; n = 135 towels)
Wyatt et al., 2002 [31]	M enstrual pictogram Symptometrics device	Women with self-perceived normal or heavy MBL	59 (109)	_	_	Overall agreement between paper assessment charts for MBL: cycle 1, 92%; cycle 2, 92%; $\kappa$ : cycle 1, 0.84 ( <i>P</i> <.00001); cycle 2, 0.81 ( <i>P</i> <.00001)
Methods involving se	lf-perception of MI	BL				
M atteson et al., 2015 [32]	MBQ	Non-pregnant women with and without self-reported HMB	27 (-)	_	MBQ domains were internally consistent (Cronbach's alpha = 0.87– 0.94)	_
			13 (-)	_	There was good-to-excellent correlation of daily, weekly, and monthly recall scores ( $\rho = 0.80-0.92$ )	_
Toxqui et al., 2014 [33]	MBL score	Healthy women	165 (-)	-	Cronbach's alpha coefficient for MBL score was 0.83	-
Rae et al., 2013 [34]	Health Utilities Index questionnaire	Women with von Willebrand disease	185 (185)	-	-	External validation (von Willebrand disease)
Bushnell et al., 2010 [35]	MIQ	Women with self-perceived normal MBL or diagnosed heavy MBL	262 (262)	_	With one exception, all intra- class correlation coefficients (reflecting test-retest reliability) for treatments and controls were $>0.70$ (n = 131)	Correlations across MIQ and to other questionnaires were almost all highly significant ( $P$ <.001)
de Souza et al. 2010 [36]	SF-36	Women with heavy MBL defined as >80 mL for $\ge$ 3 months	58 (58)	-	-	External validation (Portuguese women)
Lee et al., 2006 [37]	Question-naire (MBL, number of pads)	Adolescent girls	2411 (-)	-	-	External validation (Malaysian adolescents)

Author and year (reference)	Method	Study population	N (n) <sup>a</sup>	Recovery of blood/iron	Internal consistency	Other validation
Pawar et al., 2008 [38]	QoL questionnaire	Adolescent women	45 (-)	_	_	External validation (US adolescents)
Warner et al., 2004 [39]	MEQ	Women with self-perceived menstrual complaint	209 (N/A)	_	Compared with the previous 6 months the collected period was reported as: more in amount, 5%; the same, 16%; a bit less, 42%; much less, 36%	-
Johannes et al., 2000 [40]	A prototype electronic menstrual calendar and an identical paper calendar	Women with self-perceived normal MBL	24 (N/A)	_	_	Overall, participants preferred the electronic version (69.6% vs 21.7%; <i>P</i> =.006)
Hay nes et al. 1977 [41]	Subjective complaint	Women with self-perceived heavy MBL(25) and women with normal MBL (12)	37 (>269)	_	-	Compared to women with normal MBL there was no difference in the coefficient of variation for MBL from one period to the next

<sup>a</sup>N = study population size; n = number of cycles studied.

AH = alkaline hematin; ANOVA = analysis of variance; BQ = bleeding questionnaire; HMB = heavy menstrual bleeding; HSC = Hospital for Sick Children; MBL = menstrual blood loss; MBQ = Menstrual Bleeding Questionnaire; MEQ = Menstrual Evaluation Questionnaire; MFL = menstrual fluid loss; MIQ = Menorrhagia Impact Questionnaire; PBAC = pictorial blood loss assessment chart; SAP = superabsorbent polymer; SD = standard deviation; SE = standard deviation; SF-36 = Medical Outcomes Study 36-item Short Form Health Survey.

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