Highly Sensitive Alkaline Hematin Assay for Measurement of Menstrual Blood on Feminine Hygiene Products in Support of Clinical Trials in Women's Health

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INTRODUCTION

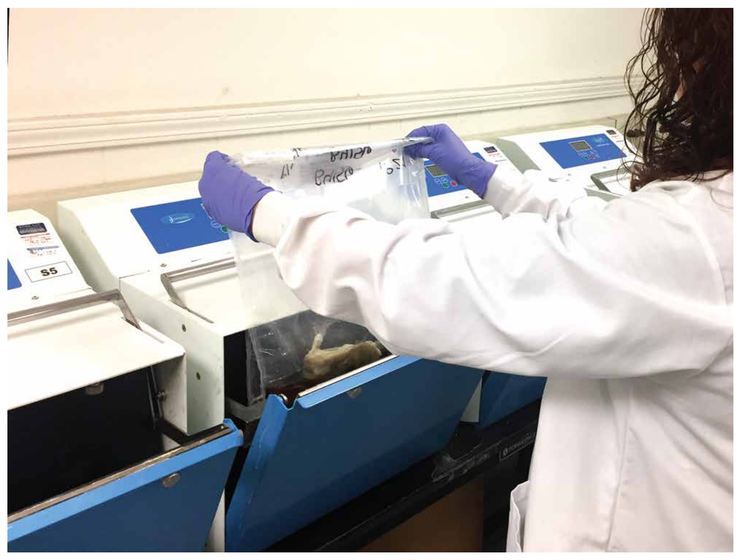
Menstrual blood loss (MBL) is often the primary efficacy endpoint in clinical trials in the treatment of idiopathic menorrhagia and in treatment of endometriosis, adenomyosis and fibroids. The two reported approaches are visual assessment (pictogram scoring, PBLAC/PBAC) and quantitative assessment via hemoglobin conversion to alkaline hematin. Pictogram assessments are typically unreliable with low correlation coefficients. To further complicate pictogram assessments, numerous product changes have recently been made by tampon and pad manufacturers that alter visual absorption patterns.

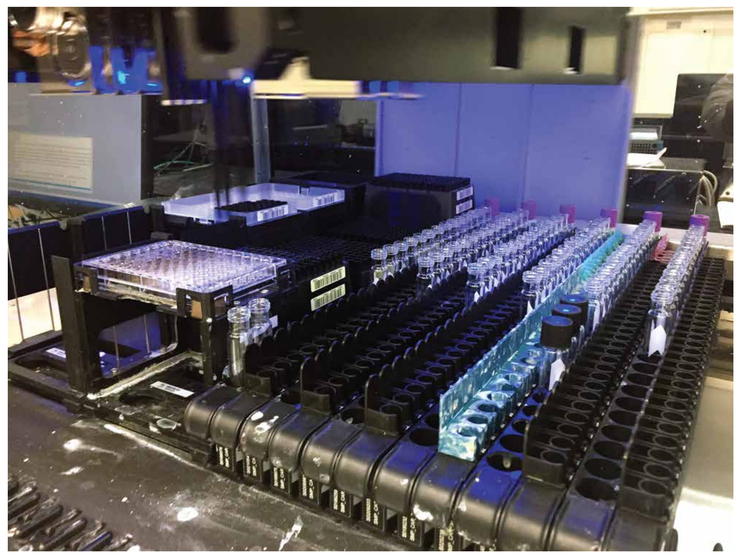
In support of these trials, our laboratory has validated a lower analytical range to be able to measure smaller blood volumes representative of spotting.

METHOD

Alkaline hematin (AH) quantitation involves pummeling used products in a concentrated sodium hydroxide solution. Under these conditions the heme moiety is released and resulting hematin can then be measured colorimetrically. Since hemoglobin levels would vary significantly over time with treatment, the method was designed using a venous blood sample from that subject for system calibration. A 10 mL K2EDTA blood sample was obtained following each menses cycle for this purpose.

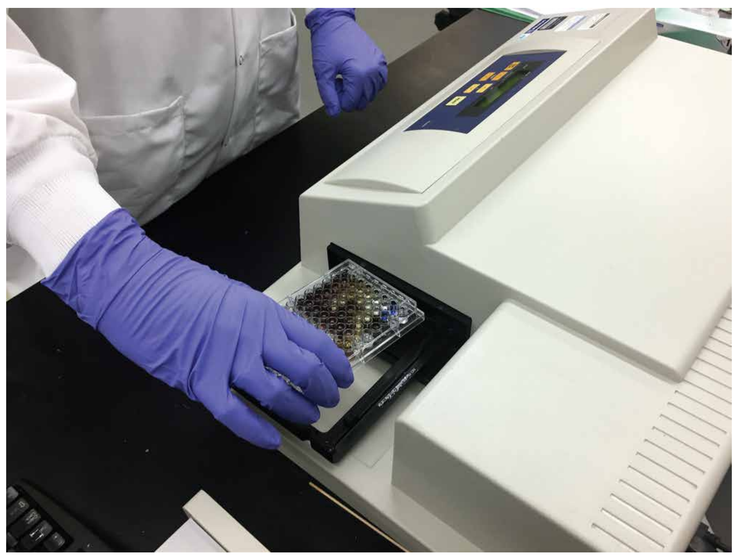
The assay was modernized using automation (Seward Stomacher®) and robotic liquid dispensing devices (Hamilton STARlet Microlab).





Products (tampons, pads and pantiliners) were evaluated individually and in combination from various manufacturers. Quality Controls samples (QCs) were prepared by placing measured amounts of blood ranging from 0.5 mL to 30 mL onto these products.

Aliquots from the extracted QC samples were placed onto a 96-well microplate, along with aliquots of the calibration standards, blanks, and controls.



VALIDATION DESIGN

- Optimal Absorbance & Kinetic Conversion to Alkaline Hematin
- Selectivity
- Linearity & Best Fit
- Precision, Accuracy & Recovery
- Dilution Integrity
- Stability
- Interference Testing

For acceptance, the precision (%CV) must be ≤ 20% at the lower limit of quantitation (LLOQ) and ≤ 15% above the LLOQ. The accuracy (% bias) must be ± 20% at the LLOQ and ± 15% above the LLOQ.

RESULTS

Selectivity

Various products from the manufacturers were evaluated for background absorbance when compared to the absorbance of the low calibration standard. A criterion of < 50% interference was employed in which several products met this requirement.

The background absorbance has not been adversely affected by the color markings on various products.

Linearity & Best Fit

System calibration of the standard response (optical density readings) was best fit using a 4-parameter, weighted regression.

Precision and Accuracy

Assay acceptance criteria was met for all product types and combination of products containing 1 mL of blood. However, acceptance criteria was not met at lower levels of blood on certain products.

Product	Inter-Assay	0.5 mL (pads, tampons) or 1 mL (liners)	1.5 mL (pads, tampons, liners) or 3 mL (combination)	15 mL	30 mL
Tampon	*Mean*	0.433	1.322	14.6	29.2
	CV [%]	11.9%	9.0%	2.4%	1.2%
	Bias [%]	-13.4%	-11.8%	-2.3%	-2.6%
Pad	*Mean*	0.0425	1.44	14.6	30.1
	CV [%]	9.9%	5.0%	1.7%	1.4%
	Bias [%]	-13.6%	-3.9%	-2.6%	0.5%
Pantiliner	*Mean*	1.06	1.55	ND	30.7
	CV [%]	7.4%	4.6%		1.7%
	Bias [%]	6.3%	3.0%		6.3%
Combination	*Mean*	1.1	3.1	15.4	31.2
	CV [%]	0.6%	0.7%	1.8%	0.7%
	Bias [%]	9.1%	2.9%	2.6%	4.0%

Tampon: Tampax Super Plus
Pad: Kotex Overnight Maxi
Pantiliner: Carefree Original Long Unscented

RESULTS (continued)

Dilution Integrity

Products containing blood volumes that exceeded the upper limits of quantitation (ULOQ) standard were diluted with sodium hydroxide solution after product extraction and analyzed directly.

Precision and accuracy data of samples diluted 1:1 and 1:3 met acceptance criteria.

Stability

Matrix stability

Whole Blood, K_2EDTA Anticoagulant

4 °C	56 days
Ambient	43 days
30 °C	55 days
45 °C	Unstable (7 days)
Blood on Product	
Ambient	≥ 57 days
30 °C	≥ 28 days *
45 °C	Unstable (7 days)

Extract stability

Alkaline Hematin

Ambient	33 hours

* Blood stability for Kotex Maxipads only demonstrated up to 7 days at 30 °C.

Interference Testing

Anticoagulant

The presence of anticoagulant is essential since calibration curves for the analysis of a given patients' samples must be prepared from the patients' own venous blood several days after collection. Freshly collected whole blood (with and without K2EDTA) was diluted in extraction solvent and the absorbances read over a 43-hr interval.

Absorbance readings were within ± 3% of each other at all time points.

Urine and Vaginal Secretions

No interferences were observed in assay performance when human urine or vaginal secretions were present on product QCs.

CONCLUSION

This higher sensitivity method has met or exceeded FDA guidance requirements and comprises one of three separate analytical ranges.

- 1 to 50 mL
- 2 to 100 mL
- 10 to 200 mL and 20 to 400 mL (with dilution)

These validated methods have been used for the determination of menstrual blood loss in over 17,000 menstrual cycles from various global feminine hygiene products. This assay has been used to support numerous clinical studies as the primary endpoint marker.