Rapid Quantitation of Menstrual Blood Loss from Feminine Hygiene Products

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INTRODUCTION

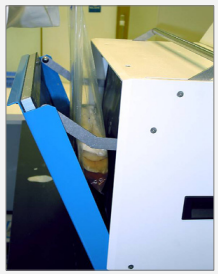
Clinical trials involving uterine sparing procedures for treatment of fibroid symptoms or drug therapies to reduce heavy menstruation require menstrual blood loss (MBL) assessment as an end point. The two reported approaches are visual assessment (pictogram scoring) and quantitative assessment via hemoglobin 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, altering visual absorption patterns. The measurement of MBL by alkaline hematin has become the method of choice and FDA's "Gold Standard" for MBL assessment.

METHOD

Alkaline hematin quantitation involves pummeling used products in a concentrated sodium hydroxide solution. Since hemoglobin values vary significantly, the method was designed measuring the resulting hematin absorbance against a calibration curve, prepared from the subjects' venous blood. A 10 mL K_2EDTA venous blood sample was required, following each subject cycle.

The method was evaluated using three types of feminine hygiene products (tampons, pads and pantiliners) from different manufacturers. Quality Controls (QCs) were prepared by placing known amounts of blood (2.5 to 30 mL) onto various products. The samples were placed into polypropylene bags, along with a specified volume of sodium hydroxide extraction solvent.

The QCs were placed into a Stomacher™ and pummeled until the extraction was complete. The resulting extracts were then incubated to permit the conversion of hemoglobin to alkaline hematin.



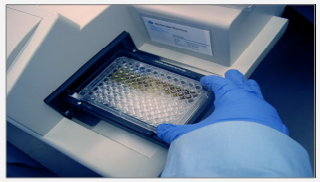
METHOD (CONT.)

Calibration Standards were prepared directly into sodium hydroxide solution with limited portions of the venous blood sample (10 mL).

A 96-well plate design was used for sample analysis. Extracted samples were aliquotted in duplicate onto the plate, along with calibration standards QC samples and solvent blanks. A solution, containing tryphan blue, was employed to monitor instrument performance throughout the study.

Spectrophotometer

Instrument:	SpectraMax 190 (Molecular Devices)
Software:	SoftMax Pro
Wavelength:	580 nm
Mode:	Endpoint



VALIDATION DESIGN

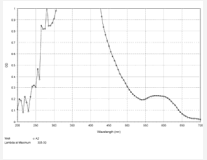
Optimal Absorbance & Kinetic Conversion to Alkaline Hematin

- Selectivity
- Linearity & Best Fit
- Precision, Accuracy & Recovery
- Blood Recovery from Products
- Dilution
- Stability
- Presence of Anticoagulant

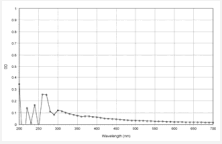
RESULTS

Absorbance Optimization

A wavelength of 580 nm was selected based on defined plateau region with minimal absorbance from blank product extracts.



Alkaline Hematin Extract

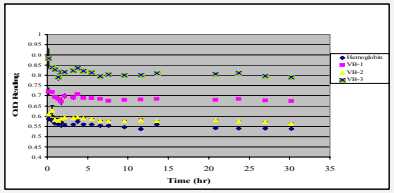


Blank Product Extract

RESULTS (CONT.)

Kinetic Conversion

The conversion of hemoglobin to alkaline hematin was evaluated a 30-hr period, using both a commercial standard of hemoglobin in water (14 g/dL) and 3 lots of human venous blood.



Conversion stabilized after 2 hours at room temperature.

Selectivity

Various products were evaluated for background absorbances across manufacturers to the absorbance of the low calibration standard. A criteria of < 50% interference was employed, in which several products met this requirement.

Selectivity was also evaluated for colorimetric interferences from various color markings on products and from human urine. Neither the new "blue striping" on redesigned products or human urine were found to interfere with the assay.

Linearity & Best Fit

The method was evaluated using two analytical ranges, 2.5 to 50 mL blood (0.5 L extraction) and 5 to 100 mL blood (1 L extraction) using six calibration levels. The data was best fit using unweighted, 4-parameter regression analysis.

Precision & Accuracy

Product	Inter-Assay	2.5 mL LLOQ	7.5 mL Low QC	15 mL Mid QC	30 mL High QC
Tampon	Mean	2.41	6.96	14.72	27.24
	CV [%]	19.4	5.8	5.2	2.8
	Bias [%]	-3.4	-7.2	-1.8	-9.2
Pad	Mean	2.68	7.38	14.23	29.00
	CV [%]	15.9	3.1	11.8	4.9
	Bias [%]	7.3	-1.6	-5.1	-3.3
Pantiliner	Mean	2.37	7.04	13.87	27.76
	CV [%]	5.2	3.4	2.9	5.6
	Bias [%]	-5.3	-6.1	-7.5	-7.5

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

RESULTS (CONT.)

Dilution

Products containing blood volumes that exceeded the ULOQ Standard were diluted with sodium hydroxide 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	≥ 56 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.

Anticoagulant

Since calibration curves for the analysis of a given patient's samples must be prepared from patient's own venous blood, several days after collection, the presence of anticoagulant is essential. Freshly collected whole blood (with and without K_2EDTA) was diluted in extraction solvent and the absorbances read over 43-hr interval.

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

CONCLUSION

This method has met or exceeded FDA guidance requirements and has been used for the determination of menstrual blood loss on over 85,000 feminine hygiene products from various clinical protocols.

ACKNOWLEDGEMENTS

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