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Supplementary information

Thrombin generation test based on a 96-channel pipettor for evaluation of FXIa procoagulant activity in pharmaceuticals

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Supplementary Information

Title: Thrombin generation test based on a 96 channel pipettor for evaluation of FXIa procoagulant activity in biopharmaceuticals

Authors: Leonid A. Parunov*, Maria E. Shea*, Yideng Liang*, Stepan S. Surov, Maitreyi Chattopadhyay, Timothy K. Lee, Dorothy E. Scott, and Mikhail V. Ovanesov

*Equal contribution

Affiliation: Center for Biologics Evaluation and Research, U.S. Food and Drug Administration, Silver Spring, Maryland, United States of America

Table S1. Description of worksheets generated by running AutoTGT

Worksheets after AutoTGT	Description
AvrThrombin	TG curves averaged across duplicates with standard deviations.
DatAMC	Fluorescence data after smoothing and drop correction and with AMC calibration applied.
DatAUC	Fluorescence data after smoothing and drop correction and with AMC calibration applied
DatRFU	Fluorescence data after smoothing and drop correction
DatThrombin	TG curves, separate for each
DatVelocity	Velocity taken from TG curves
RawData	Is not used in this version of script
ResultsTG	Thrombin parameters averaged across duplicate wells
ResultsTGall	Thrombin parameters separate for each well
USERcalibrDT	Is not used in this version of script
WellDataFG	Is not used in this version of script
WellDataTG	Enter fluorescence data here for thrombin generation
WKSHTResults	Is not used in this version of script
Temp*	Temporary worksheets

Table S2. Description of worksheet columns in ResultsTG. Note that the SD suffix refer to the reported values standard deviation when there are duplicate wells for a sample.

ResultsTG Column Heading	Description
NSample	Sample number
SampleName	Sample name
totalTG.1 (TG.1'SD)	AUC (area under the curve) up to first time cutoff (default 20 minutes)
totalTG.2 (TG.2'SD)	AUC up to second time cutoff (default 40 minutes)
totalTG.3 (TG.3'SD)	AUC up to third time cutoff (default 60 minutes)
totalTG.4 (TG.4'SD)	AUC up to fourth time cutoff (default 90 minutes).
maxTG (maxTG.SD)	Maximum thrombin generation, used to calculate potency TPH (thrombin peak height).
TimemxTG (TimemxTGSD)	Time of maximum thrombin generation, (time to thrombin peak).
maxTGrate (maxTGrateSD)	Maximum rate of thrombin generation, (velocity).
timeMaxTGrate (tiMaTGSD)	Time of max TG rate, (time to maximum velocity)
Time.10nM (Time.10nMSD	Time to 17% of thrombin generation curve (default), referred to as lag time, LagTime or LT later

Table S3. Description of worksheet columns in ResultsTGall. Note that .avr and .SD suffixes refer to the averaged values and the average's standard deviation.

ResultsTGall Column Heading	Description
SampleN	Sample number
SampleName	Sample name
AUC.1 (AUC.1.avr, AUC.1.SD)	AUC (area under the curve) up to first time cutoff (default 20 minutes)
AUC.2 (AUC.2.avr, AUC.2.SD)	AUC up to second time cutoff (default 40 minutes)
AUC.3 (AUC.3.avr, AUC.3.SD)	AUC up to third time cutoff (default 60 minutes)
AUC.4 (AUC.4.avr, AUC.4.SD)	AUC up to fourth time cutoff (default 90 minutes). This value is used as the AUC value for the activity calculation
TpeakHeight (TGpeakH.avr, TGpkMax.SD)	Maximum thrombin generation, used to calculate activity using TPH (thrombin peak height)
TtoPeak (TtoPeak.avr, TtoPeak.SD)	Time of maximum thrombin generation, used to calculate activity using TTP (time to thrombin peak)
VelocityMax (VelocMx.avr,VelocMx.SD)	Maximum rate of thrombin generation, used to calculate activity using VEL (velocity)
TtoVMax (TtoVmax.avr, TtoVmax.SD)	Time of max TG rate, used to calculate activity using TVL (time to maximum velocity)
LagTime (LagTime.avr, LagTime.SD)	Time to 17% of thrombin generation curve (default) used to calculate activity using LT (lag time)

Table S4. Description of worksheets generated by running aPotency.

Worksheets after running aPotency	Description
PotencyALL	Activities of each sample calculated using different parameters (PH, TTP, LT, AUC, VEL, and TVL) are combined in one worksheet.
PotencyAUC	Activity calculations using area under the curve (AUC at 90 minutes, AUC.4)
PotencyLT	Activity calculations using lag time
PotencyTPH	Activity calculations using thrombin peak height
PotencyTTP	Activity calculations using time to peak
PotencyTVL	Activity calculations using time to maximum velocity
PotencyVEL	Activity calculations using maximum velocity
SerialDilAUC	Area under the curve values for plotting
SerialDilLT	Lag time values for plotting
SerialDilTPH	Thrombin peak height values for plotting
SerialDilTTP	Time to peak values for plotting
SerialDilTVL	Time to maximum velocity values for plotting
SerialDilVEL	Maximum velocity values for plotting

Table S5. Description of graphs generated by running aPotency.

Graphs after running aPotency	Description
_PotencyALL	Activities of each sample calculated from TPH, TTP, LT, AUC, VEL, and TVL parameters plotted as a bar graph.
_SerialDilALL	Parameter values of each sample plotted against the sample's dilution fold. Points are masked if excluded from activity analysis.
_SerialDilAUC	Area under the curve values of each sample plotted against the sample's dilution fold.
_SerialDilLT	Lag time values of each sample plotted against the sample's dilution fold.
_SerialDilTPH	Thrombin peak height values of each sample plotted against the sample's dilution fold.
_SerialDilTTP	Time to peak values of each sample plotted against the sample's dilution fold.
_SerialDilTVL	Time to maximum velocity values of each sample plotted against the sample's dilution fold.
_SerialDilVEL	Maximum velocity values of each sample plotted against the sample's dilution fold.
_StdCurvesAll	Parameter values of each Standard and the fitted standard curve plotted against the Standard's dilution fold. Masking points on this graph and refreshing the standard curves will exclude these points from fitting.
_TGcurves[*your Standard Lot number*]	Thrombin generation curves for the standard are plotted.
_TGcurvesALL	Thrombin generation curves for the standard and the samples are plotted side-by-side.
_TGcurvesSmpl1	Thrombin generation curves for the samples are plotted together.

Table S6. Description of worksheets columns in PotencyAUC.

These column descriptions are the same for the PotencyLT, PotencyVEL, PotencyTTP, PotencyTPH, and PotencyTVL worksheets

PotencyAUC (TPH,TTP, etc.) Columns	Description
Concntrtn	Concentration of standard
StdCurve (StdCurveSD)	Parameter value at given concentration of standard
ConcCorr	Log10 of concentration of standard
StdCurveCorr	Value of standard at Conccorr. Depending on settings, background may be subtracted, and log transformation applied
SDCorr	Standard deviation of StdCurveCorr. Depending on settings, background may be equal to StdCurveSD or corrected when background is subtracted (calculated as standard deviation of two random variables).
StdCurveLog	Log transformed StdCurveCorr
Residual	Residual from fit (difference between fit and observed value)
RelativeResi	Relative residual (ratio of residual to observed value)
ConcFit	Concentration of standard for fitted curve. Depending on settings may be expressed in linear or logarithmic values.
StdCurveFit	Value of fitted standard curve at ConcFit
FitParameter	Fit parameter names
FitParValue	Fit parameter values
YStd	Concentration of standard for all points on standard curve. Depending on settings may be expressed in linear or logarithmic values.
XStdSignal	Value of fitted standard curve at given YStd
YStdLogC	Log10 of concentration of standard for all points on standard curve

YStdConc	Concentration of standard for all points on standard curve
XStdSignalR	Reverse transformation from logarithmic to linear scale for XStdSignal
WellN	Number of well
WellName	Name of well
WellValue	Parameter value for given well
WellPotency	Calculated activity (or potency) (unadjusted) for given well
WellDilution	Dilution fold for given well
WPotByDil	Activity multiplied (WellPotency) by dilution fold (WellDilution) for given well
SampleN	Number of sample
SampleName	Name of sample
NReplicate	Number of replicates for each sample
SampleAvr	Average value for the parameter of sample
SampleSD	Standard deviation of the parameter of sample
SPotencyAvr	Averaged activity for each sample
SPotencySD	Standard deviation of the activity for each sample
LotDilN	Number of sample
LotNumber	Name of lot (pooled samples of different dilutions)
Dilutions	Dilution factor for each sample
PotByDil	Averaged activity (SPotencyAvr) multiplied by dilution fold (Dilutions)
LotName	Name of each lot
POTENCY	Activity averaged for all dilutions in a lot
POTENCYSD	Standard deviation of averaged activity
CV	Coefficient of variation (standard deviation/average activity)
N	Number of values (dilutions) averaged to find the activity

Table S7. Description of worksheet columns in PotencyALL. Note that the SD suffix refer to the reported activity's standard deviation.

PotencyALL Column Heading	Description
LotName	Name of Sample Lot
TPH (TPH.SD)	Activity calculated using thrombin peak height
TTP (TTP.SD)	Activity calculated using time to peak
LT (LT.SD)	Activity calculated using lag time
AUC (AUC.SD)	Activity calculated using area under the curve
VEL (VEL.SD)	Activity calculated using maximum velocity
TVL (TVL.SD)	Activity calculated using time to maximum velocity
Avr (Avr.SD)	Average activity across all parameters