A novel prothrombin time reagent that utilises tissue factor generated by silkworm-baculovirus technology

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Introduction

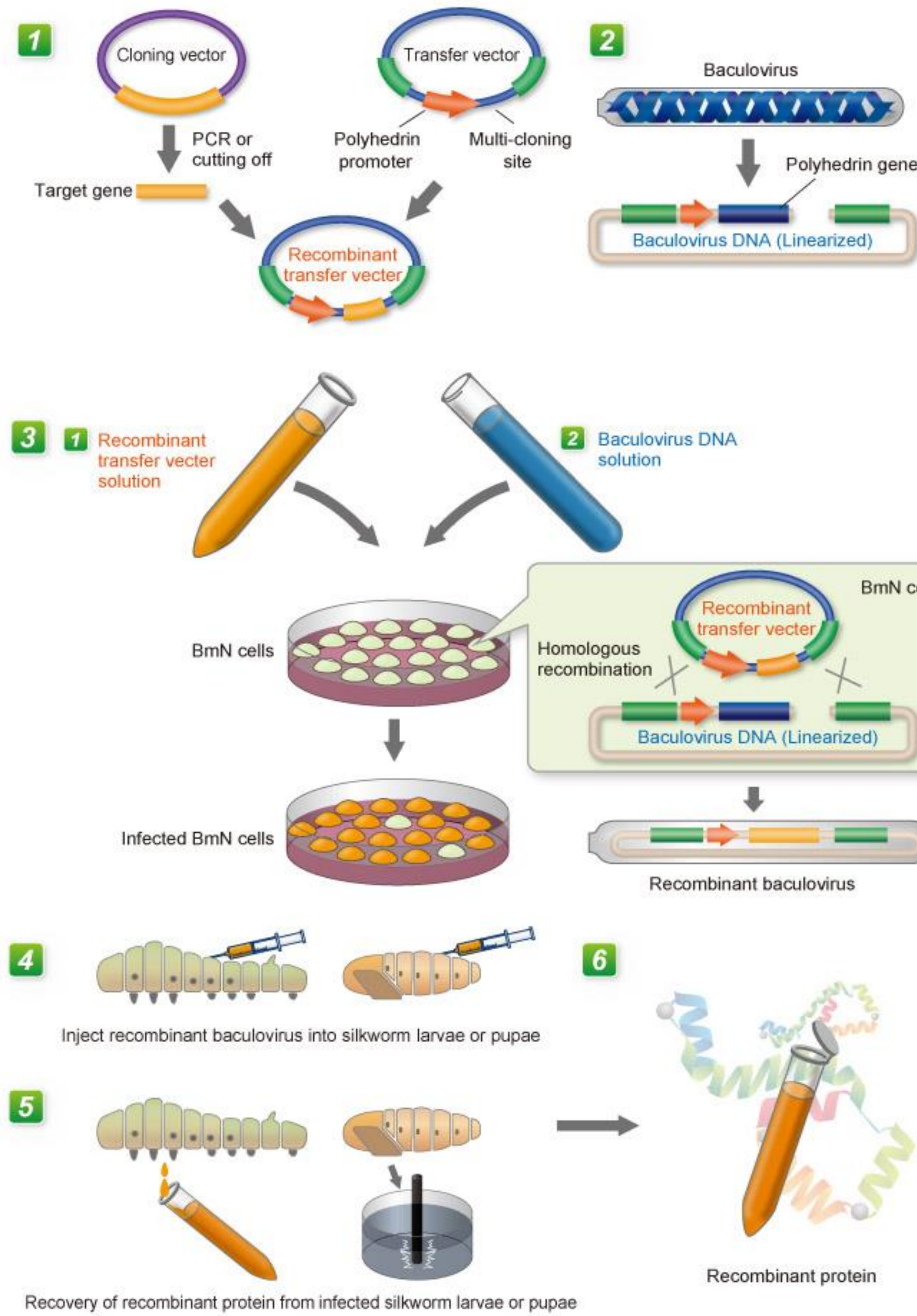
The Silkworm-Baculovirus (sb) expression system for recombinant proteins has several advantages over E. coli, e.g., increased yield, improved solubility and incorporation of post-translational modifications (e.g., glycosylation). A novel prothrombin time reagent (sbPT, Sysmex Corporation. Japan) has been developed using recombinant human tissue factor produced by this system (Fig. 1).

Fig. 1Protein Production

Construction of transfer vector

The recombinant transfer vector is co-transfected with the baculovirus DNA into silkworm cells (BmN).

The recombinant baculovirus is injected into a larva or pupa. The hemolymph or tissue are harvested. The protein is purified from soluble fractions using standard chromatography columns.



The aim of this study was to compare the performance of sbPT with two widely used PT reagents.

Methods

The performance of sbPT was compared to two commercial PT reagents containing either human placental thromboplastin (PTA) or recombinant human TF (PTB) on a Sysmex CS-5100 coagulometer, using instrument-specific ISI (1.00, 1.07 and 0.97 for sbPT, PTA and PTB respectively). On-board stability and imprecision were assessed using commercial QC preparations. Comparability testing was performed on over 300 normal and abnormal plasma samples.

Results

sbPT demonstrated excellent levels of imprecision (Table 1) and on-board stability (less than 2.2% variation over 4 days). Normal reference ranges of 9.8 – 12.8s (sbPT), 10.4 – 13.6s (PTA) and 9.6 – 11.5s (PTB) were established in plasmas from 100 normal healthy donors.

Table 1. Between day imprecision

	Normal QC	Abnormal QC
Mean	12.4 s	36.1 s
SD	0.10	0.30
CV	0.81%	0.83%

INR values for 130 plasma samples from patients receiving warfarin showed good agreement between methods (Figures 2 and 3; mean INR 3.06; 3.09 and 3.02 for sbPT, PTA and PTB respectively. Most INR were within 0.5 INR units (99% for PTA and 96% for PTB). All three PT methods exhibited similar sensitivity to FII deficiency, while sbPT was more sensitive to deficiencies of FV, FVII and FX and coagulation defects in liver disease.

Fig. 2. INR values for normal controls (n = 100) and patients receiving warfarin (n = 130). PTA vs sbPT

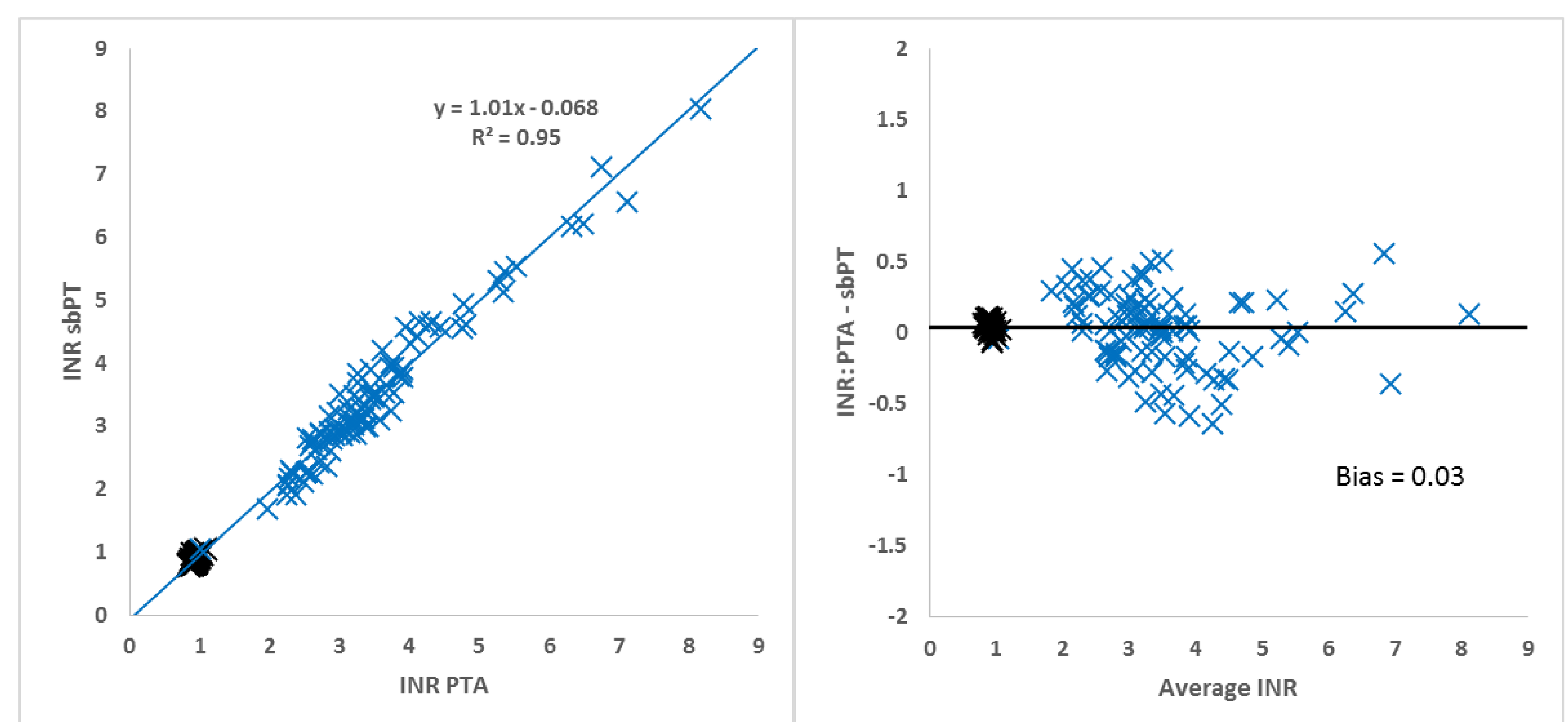
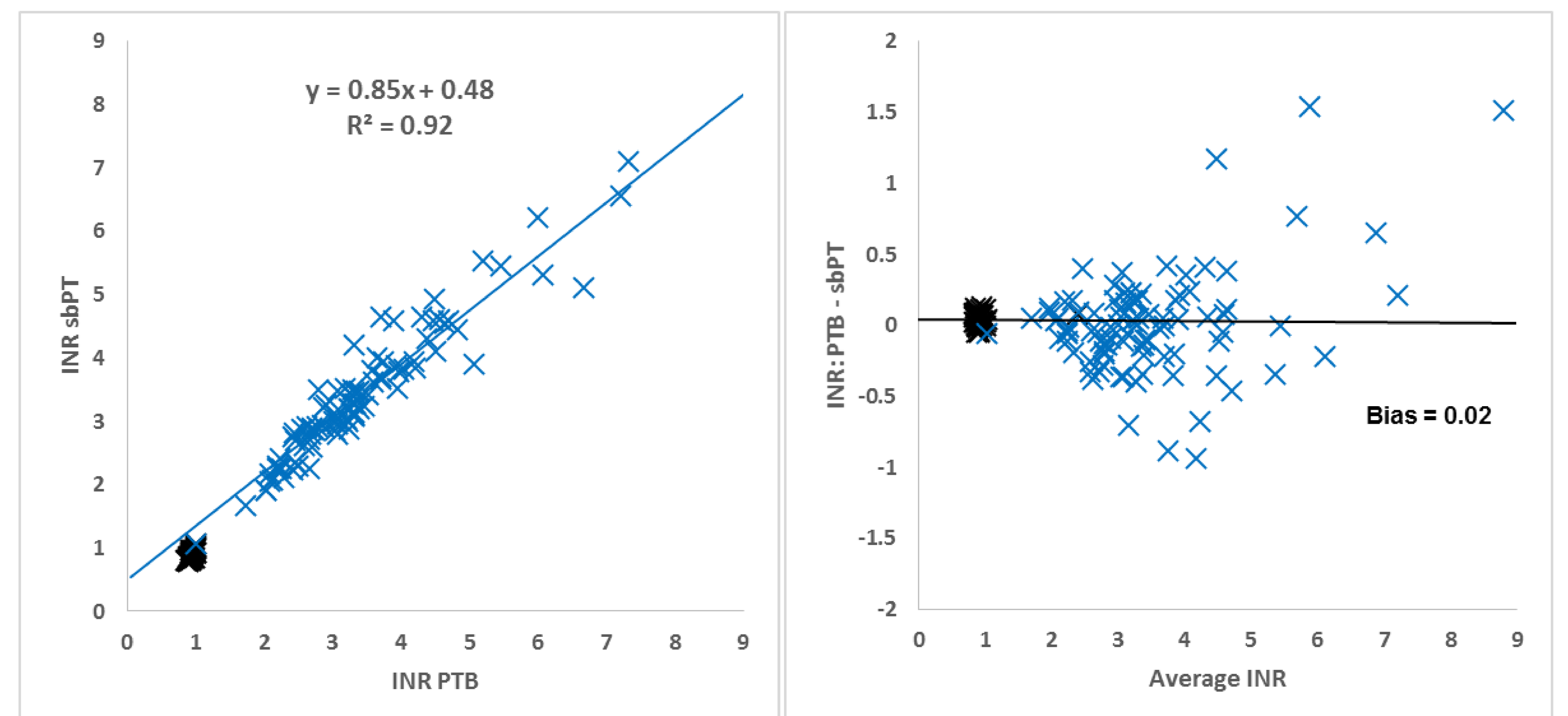


Fig. 2. INR values for normal controls (n = 100) and patients receiving warfarin (n = 130). PTB vs sbPT



Heparin interference was not detected by sbPT or PTB (both of which contain a heparin neutralising compound) at levels up to 1.5 IU/mL. All three reagents had similar sensitivity to lupus anticoagulant. sbPT demonstrated dose responsiveness to Rivaroxaban, Dabigatran and Apixaban and with steeper response curves than PTA or PTB.

Conclusions

In a wide range of plasma samples, sbPT demonstrated comparable or improved performance relative to two commercial PT reagents which are suitable for the control of oral vitamin K antagonist therapy and the detection of congenital or acquired deficiency of FII, FV, FVII and FX.