Clinical Case Study

A Febrile Blood Donor

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CASE DESCRIPTION

A 4-day-old neonate born prematurely at 29 weeks gestation developed thrombocytopenia (platelet count 43 x 10^9/L) with associated severe pulmonary and intracranial hemorrhaging. Urgent transfusions with 10 mL/kg of packed red cells and 10 mL/kg of platelet concentrate were given. These were sourced from blood donated 2 days earlier in Singapore by a 21-year old female university student who was clinically well at the time. The day after these blood products were given to the neonate, the blood donor contacted the blood transfusion service to inform them that she was now sick with a febrile flu-like illness. Her blood donation screening plasma sample was retrieved and tested by PCR for several viruses, including dengue, chikungunya, enterovirus, and Epstein-Barr virus.

Dengue RNA (serotype 2) was found to be present at a very low amount (i.e., at a real-time PCR cycle C_t number of 39–40) (Fig. 1A) from the initial RNA extract (extract A). To confirm the presence of this low amount of dengue 2 RNA, the same extract (extract A, which had been stored at -20 °C) was retested (Fig. 1B), and again found to give a low-positive result.

Questions to Consider
• What steps should be taken next?
• What can cause false-negative and false-positive PCR results?
• How should low-positive PCR results affect patient management?

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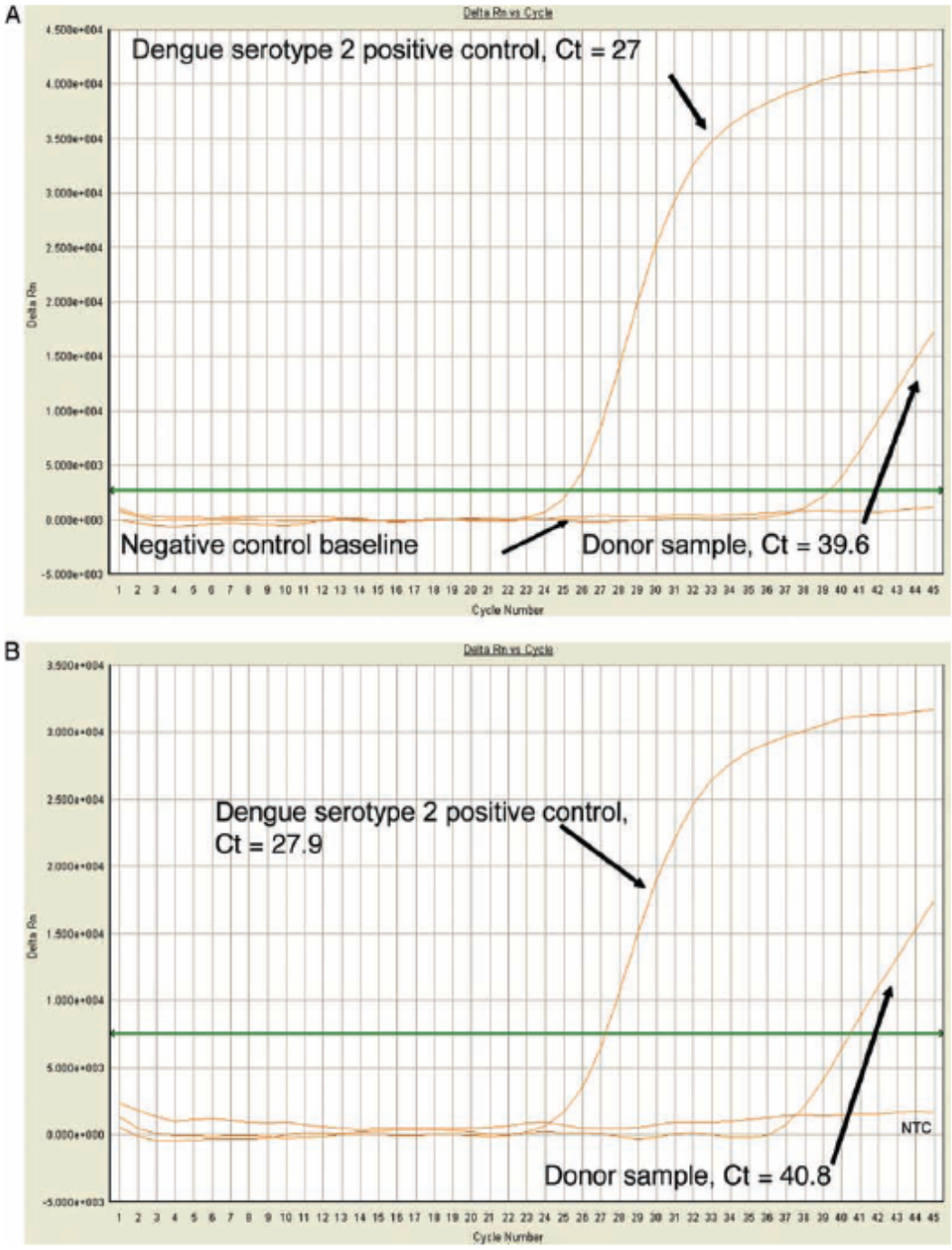

Fig. 1. Real-time dengue RNA PCR outputs for the donor plasma sample.

(A), Real-time PCR results on the RNA extract (extract A, first test) obtained from the first donor sample showing a Ct of 39.6, a low-level positive signal for dengue serotype 2 RNA. (B), Repeat testing on extract A after 1 cycle of freeze-thawing (from −20 °C), showing a higher Ct of 40.8, indicating an even lower amount of RNA present. The normal real-time PCR output is seen as a sigmoidal plot. The Ct value is where the signal sigmoid curve (indicating accumulation of the specific dengue PCR product) crosses the threshold (green horizontal line) indicating a positive result. The threshold for individual curves may be different and can be automatically set by the assay software, but can also be adjusted according to local dengue virus epidemiology and the expected Ct values for positive results in this specific patient population.

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Final Publication and Comments

The final published version with discussion and comments from the experts will appear in the March 2010 issue of *Clinical Chemistry.* To view the case and comments online, go to http://www.clinchem.org/content/vol56/issue3 and follow the link to the Clinical Case Study and Commentaries.

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