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**Letter in Reply**

Reusable blood collection tube holders are implicated in nosocomial hepatitis C virus transmission

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*To the Editor—*In response to the letter by Tsang et al, we offer the following point-by-point rebuttal. Almost all hospital outbreak investigations require direct observation of patient care practices that are confounded by the Hawthorne effect, leading to the underestimation of incorrect practices. Despite this limitation, our direct observation showed that our phlebotomists had not been trained to comply with 2 manufacturers’ instructions: (1) the need for disinfection of reusable blood collection tube holders (RBCTH) between every patient and (2) the release of tourniquet immediately after blood starts flowing into the specimen tube to minimize backflow.1–3 These noncompliant practices had been adopted by all phlebotomists since the introduction of these 2 RBCTH brands into Hong Kong public hospitals.

The male source patient and the female victim were housed in the same ward served by 1 or more phlebotomists. Our computerized barcoding system recorded 34 phlebotomists with 54 visits to this ward for all patients between August 6, 2017, and August 19, 2017. The same phlebotomist collected blood from the source patient before collecting blood from the female victim in the morning shift on August 9, 2017, (phlebotomist A) and on August 11, 2017 (phlebotomist B). Of 29 phlebotomists being interviewed, 28 reported the sole use of RBCTH kept in the ward’s phlebotomy trolley where the HCV-positive RBCTH was found, including those who provided services on August 9, 2017, and August 11, 2017. Because HCV remains infectious for 6 weeks in the environment, patients were at risk of exposure whether the same or different phlebotomists took blood from the victim before or after the source patient, as long as the HCV-contaminated RBCTH in this phlebotomy trolley was in use. Using a barcoding system, we reviewed an earlier case of nosocomially acquired HCV in a 94-year-old female (supplementary material online). A phlebotomist took blood from a 78-year-old female HCV-positive patient on April 7, 2016, and collected blood from this victim immediately afterward. Again, the RBCTH could have been the vector; extensive investigation did not identify any other modes of transmission.

In addition to the RBCTH, only 1 glucometer and 1 phlebotomy trolley in use could be sampled for HCV on December 5, 2017. Our direct observation showed no practice irregularities in venous catheter insertion or multiple-dose drug-vial sharing. The presence of the same HCV genotype with almost identical genetic sequences inside the RBCTH and from the source patient and the victim clearly demonstrated that the phlebotomists had used this RBCTH and that blood contamination inside this RBCTH had occurred. Therefore, the possibility of both source and victim sharing this RBCTH has to be entertained.

Until now, none of the reported HCV hospital outbreaks have utilized whole-virus genome sequencing, although partial-genome quasi-species sequencing was advocated by Campo et al.4 Most previous reports used the hypervariable region (E1 and E2 HVR), with only 140 bp to 411 bp sequenced.5,6 Others have used the NS5b, with 328 bp sequenced.7 Only 1 study has evaluated environmental samples using 81 bp of HVR between patients and environmental samples. In this case-control study suggesting the sharing of multiple-dose heparin vial as the source, no HCV could be detected in these vials.8 Another case-control study using multivariate analysis showed that international normalized ratio (INR) monitoring by phlebotomy and podiatry were risk factors for HCV acquisition,9 and next-generation sequencing was used to analyze 291 bp of HVR quasi-species instead of the entire genome. In this study, <3% (9 bp) of the genome was used as evidence of clonality between HCV strains.9 Thus, none of the phylogenetic studies of HCV outbreak were as stringent as ours. With only 3 nucleotide positions of divergence of 653 bp (HVR) between the HCV sequences from the RBCTH, the source patient, and the victim, we thus confirmed clonality.

As for the phlebotomy simulation experiments, we used only HCV-positive plasma because no HCV-positive archived EDTA blood was available. Blood is denser and more adhesive than plasma, which would lead to a larger volume of inoculum remaining on contaminated surfaces. The dipping of 5 mm of the tip of a rubber sleeve picked up 0.06 μL (mean±SD, 0.02 μL) of the HCV-positive plasma. This volume was so low that this inoculum was invisible to the naked eye and was less than the 1.4 μL (mean volume) inoculum from a hollow-needle needlestick injury.10 Therefore, this procedure was a reasonable substitute for in vitro simulation.

The objective of the 2 radionuclide experiments was to dynamically investigate the possibility of reflux communication from RBCTH into the patient during a simulated phlebotomy (not virus transmission). A reflux can only happen in 2 conditions: bidirectional patency and pressure difference. Blood has both solution and colloidal properties; therefore, it is a homogeneous carrier medium for transport of all its normal constituents (from cells to molecules). Ideally, 99mTc-labeled blood (eg, used for study of gastrointestinal bleeding) could be used for this experiment. In addition, a 16G–20G needle has an inner cross-sectional area 7,000–27,000 times that of red blood cells, which is thus statistically and obviously not a limiting factor for the size of a molecule or virus. The pressure change created by releasing the manual pressure from the saline bag was unquantified (please note that we did not say negative pressure). However, during phlebotomy, differential pressure changes are known to be operator and patient dependent; therefore, they vary and, likewise, were unquantified. Our experiment clearly demonstrated that even a very gentle manually applied dynamic pressure difference on the saline bag could induce a patent route of reflux from the needle side to the bag side.

Regarding the backflow of blood, the crux of the matter is the sudden release of the tourniquet, which allows the venous blood under positive pressure below the tourniquet (at antecubital fossa level) to go above the tourniquet at arm level. Thus, the HCV-contaminated “blood pool” between sleeved needle and the sleeve flows back into the patient. Thus, creating negative pressure in the venous system is unnecessary for causing the backflow. As long as there is a pressure gradient between the vein below and above the tourniquet, the HCV-contaminated blood can flow into the patient when the tourniquet is released.

We did not find any peer-reviewed journal, publication-quality methodology or data in reference 5 cited by Tsang et al. This citation refers to the evaluation of a single-use tube holder and the low risk of backflow from the vacuum specimen container through the sleeved needle back into the patient, whereas our study refers to RBCTH and the risk of backflow from the blood pool between the sleeve and the sleeve needle (not the vacuum specimen tube) into the patient. Both our testing with HCV-positive plasma (with virions much bigger than technetium) and radioactive technetium (visible on radiation scanning) showed significant backflow into the patient’s side, therefore posing a risk to the patient. Most importantly, our phlebotomists have not been trained to comply with the manufacturer’s instruction of the need to release the tourniquet once the blood starts to flow into the vacuum specimen tube.

Without providing evidence that the blood inside the tube holder belonged to the patient’s own blood and not the source patient, the opinion of Tsang et al must be considered speculation. We showed that the genetic sequences from the source, the RBCTH, and the victim were 99.54% identical.

We believe that the evidence presented in our original article and our present rebuttals are scientifically well grounded, contrary to the description by Tsang et al as “exaggerated, flawed, superfluous, hasty, premature or disproportionate.” Our data were scrutinized by the Hospital Authority governing all public hospitals in Hong Kong. The Hospital Authority terminated the further use of RBCTH to protect patients. We now follow the best practice in the United States, United Kingdom, and Australia of using only disposable single-use tube holders. If RBCTH are ever used, the phlebotomist must comply with the manufacturers’ instructions by disinfecting all RBCTH between patients and by releasing the tourniquet once blood starts to flow into the specimen containers. Unfortunately, these important instructions have not been provided to frontline healthcare workers for many years; thus, more cases presenting with HCV cirrhosis and hepatocellular carcinoma may be expected over time.

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