In reply: Blu-ray beyond music and movies—novel approach to diagnostics measuring specific extracellular vesicles

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We are grateful to Kristensen *et al.* (1) for reviewing our study describing a novel approach to diagnostics measuring specific extracellular vesicles (exosomes), called ExoCounter, based on optical disc and nano-bead technology, which was recently published in *Clinical Chemistry* (2).

The authors emphasized the potential of ExoCounter as a method to detect and quantify specific exosomes, and they pointed out several concerns regarding the characteristics of our equipment. One of the concerns regarding detection with ExoCounter was its performance on samples with considerably high concentrations of exosomes, such as in diseased states. The ExoCounter can detect a range of concentrations of exosome particles (1×10^3/L to 5×10^5/L) with high linearity (*Figure 1*). However, as they have pointed out, at high concentrations of exosomes (7×10^5/L, *Figure 1*, marked in circle), the number of exosomes detected was slightly decreased compared with the linearity line for the detection. This may occur because some exosomes on the disc are closely bound to other exosomes, or due to an increase in the number of single nano-beads that bind two exosomes. Therefore, if the concentrations were significantly high, an appropriate dilution would be necessary for the precise detection of exosomes. Regarding the concentration of exosomes in diseased states, the number of the CD9/CD63-positive ubiquitous exosomes in sera, irrespective of whether they were derived from healthy donors or diseased patients, was $0.5–2\times10^5$/L, which was within the linear range (2). Furthermore, the number of disease-specific exosomes was considerably smaller than that of total exosomes. Furthermore, we showed that the concentration of the cancer-specific exosomes such as CD147- or HER2-positive exosomes ($0.1–1\times10^4$/L) was less than that of the CD9/CD63-positive ubiquitous exosomes in cancer patient sera (2). Thus, we believe that the ExoCounter adequately covers the measurement range required for the clinical samples.

The authors have also pointed out the lack of unique markers of cancer exosomes for cancer diagnosis. While we showed that the HER2-positive exosomes specifically increased in exosomes derived from ovarian or breast cancer patients, it is well-known that 25–30% of HER2 overexpression is found in invasive breast cancers, and its expression is an important factor in determining the treatment regimen, such as Herceptin for targeting HER2 (3). Therefore, further studies using fresh well-characterized blood samples of cancer patients are necessary for utilization in specific diagnostic uses. In addition, the study will be aimed at improving diagnostic accuracy by combining the detection of HER2 exosomes and other types of specific exosomes. This type of multiple-marker detection with the ExoCounter can be performed by preparing varied antibody-sets (sets of disc and nano-bead antibodies) for each well of the disc. Thus, these analyses would provide a novel insight into the development of precise diagnostics or determination of treatment regimen for breast or ovarian cancer patients. Finally, the ExoCounter is commercially

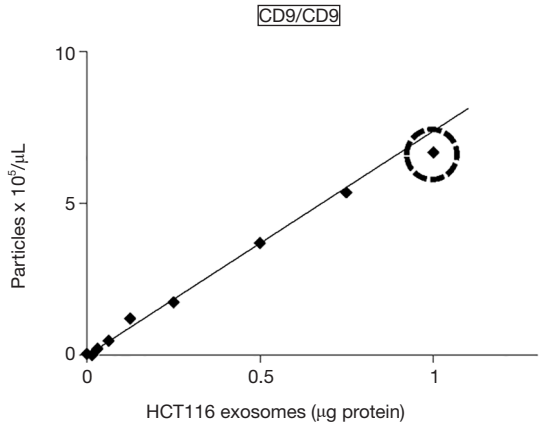

Figure 1 Quantification of exosomes using the ExoCounter. HCT116-exosomes were incubated on discs coated with anti-CD9 antibody, and subsequently treated with anti-CD9 antibody-conjugated nano-beads (FG beads).

available from JVCKENWOOD Corp. (http://healthcare.jvc.com/exosome/).

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Footnote

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Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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