Discussion about staining of “rods and rings (RR)” in Fluoro HEPANA test (HEPANA)

In our sample staining service for the customers using HEPANA, there are specific patterns which look like “rod-type” and “ring-type” fluorescent staining in a cytoplasm. These are called “cytoplasmic rods and rings (RR)” as reported by several papers. In recent knowledge, it became clear that RR is detected specifically in serum of patients with chronic hepatitis C. Among them, RR is detected more frequently in hepatitis C patients receiving combination therapy of pegylated Interferon (PEG-IFN) and ribavirin (RBV). It also seems that so far, there are no reports that RR was detected in patients without any treatment. The current study reports CTPS1 and IMPDH2 were identified as components associated with RR.

CTPS1 and IMPDH2 play role in the biosynthetic pathway for CTP and GTP, respectively, which are as nucleotide sources for DNA/RNA. It is known that RR appears when the CTP or GTP synthetic pathway is inhibited, and it is also reported that RR formation was observed even when HEp-2 cells themselves were treated with CTPS1 or IMPDH2 inhibitor. The ribavirin is an IMPDH2 inhibitor, so that RR may be formed in hepatitis C patients who were actually treated with ribavirin. If the formed RR was released somehow into the blood, the immune system would recognize it as a foreign substance to induce autoantibodies to RR. This is the assumed mechanism of RR staining detected by sera from patients with hepatitis C. As possible mechanisms for formation of RR in HEp-2 cells, it is reported that RR was formed by not only inhibition of CTP and GTP synthetic pathway, but also other conditions, such as nutrient starvation (lack of glucose), addition of sodium azide, and treatment with some kinds of kinase inhibitor. In case of our HEPANA, MBL has experience that the frequency of RR formation is dependent on the lot of the calf serum used for HEp-2 cell culture. Such lot-to-lot variation may provide different nutritional conditions to form RR. In fact, HEp-2 growth rate seems to be variable according to the lot of calf serum. Together with, we assume that when HEp-2 cells are exposed with a certain lot of calf serum which tend to provide a starvation, RR formation would be induced in the cells.

[reference 76-80]