Automated patch-clamp systems have dramatically improved efficiency of the hERG assay to detect drugs’ potency of QT-interval prolongation that may lead to induction of life-threatening ventricular arrhythmia, torsades de pointes, and cardiac sudden death. However, it has become clear that the experimental results of some of known hERG-channel blockers in the automated patch-clamp system differ from those obtained in the manual patch-clamping. Concentration-response curves of the hERG-channel blockers obtained in the automated patch-clamp system shifted in the higher-concentration direction compared to those obtained in the manual patch-clamping. This fact may affect the reliability of the hERG assay using the automated patch-clamp system, because the potency of a compound in inhibiting hERG currents could be underestimated.

In this study, we investigated the possibility of 4 known hERG-channel blockers to adsorb into 96-well plates that are used to store test-substance solutions. Samples were taken at the respective time points and these sampled test solutions were thoroughly mixed with 200 μL of methanol, and their concentrations were determined by HPLC assay. Then, the results were compared between 5 types of plates; polystyrene, polypropylene, glass, glass-coated plastic, and hydrophilic-coated plastic. Test solutions were prepared as follows: First, the test substance was dissolved in dimethyl sulfoxide to yield concentrations of 100, 300, and 1000 μM. These solutions were then diluted 1000 times with the extracellular superfusing solution. A total of 300 μL of the test solutions at the respective concentrations were placed in wells of the 96-well plates; then, 200 μL of each of the test solutions was sampled with time. A total of 8 sampling time points, ie, before placing in wells, 0, 5, 10, 15, 30, 45, and 60 minutes after placing, were set. Three samples were taken at the respective time points and these sampled test solutions were thoroughly mixed with 200 μL of methanol, and their concentrations were measured using HPLC (Hitachi High-Technology Corporation).

The time course of concentrations of 4 known hERG-channel blockers, haloperidol, E-4031, thioridazine and astemizole, in test solutions were determined by HPLC assay. Then, the results were compared between 5 types of plates, polystyrene, polypropylene, glass, glass-coated plastic, and hydrophilic-coated plastic. Test solutions were prepared as follows: First, the test substance was dissolved in dimethyl sulfoxide to yield concentrations of 100, 300, and 1000 μM. These solutions were then diluted 1000 times with the extracellular superfusing solution. A total of 300 μL of the test solutions at the respective concentrations were placed in wells of the 96-well plates; then, 200 μL of each of the test solutions was sampled with time. A total of 8 sampling time points, ie, before placing in wells, 0, 5, 10, 15, 30, 45, and 60 minutes after placing, were set. Three samples were taken at the respective time points and these sampled test solutions were thoroughly mixed with 200 μL of methanol, and their concentrations were measured using HPLC (Hitachi High-Technology Corporation).

The influences of the adsorption of astemizole on the concentration-response for the hERG-current inhibition were investigated by the whole-cell patch-clamp technique using the automated patch-clamp system, PatchExpress 7000A (Molecular Devices Corporation). Differences in the hERG-current-inhibition level of astemizole at 10, 30, and 100 nM with the aforementioned 5 types of 96-well plates were assayed.

The time course of concentrations of 4 known hERG-channel blockers in test solutions stored in 5 types of plates were determined by HPLC assay. The concentrations of a test-substance in the test solution were plotted as a function of durations to keep the solution in the 96-well plate.