

Challenging the Challenges of Nanoparticle Testing Using *In Vitro* Methods

Nashwa Osman¹, Darren Sexton¹ & Imran Saleem¹

¹Liverpool John Moores University, School of Pharmacy and Biomolecular Sciences
Byrom Street, Liverpool, L3 3AF, UK

Introduction: *In vitro* testing using cell cultures are the cornerstone standard methods for the initial screen of nanoparticles (NPs) providing many valuable mechanistic evaluations for NPs interactions with the target tissues. For example, Alamar Blue (AB), Lactate Dehydrogenase assay (LDH), Reactive Oxygen Species (ROS) assays are widely used assays for evaluating NP toxicity testing. However, these methods were adopted from the conventional chemical materials toxicology testing where these materials are soluble in the testing medium. The particulate nature and the exceptional NP physicochemical characteristics influence completely their behaviour under *in vitro* testing that render false positive or false negative results. Our study will illustrate technical issues that can be seen in *in vitro* testing in commonly used assays such as AB, LDH, and ROS. **Methods:** Assays were performed according to standard protocols or adapted by applying procedures to minimize the NPs-interactions with the assays. **Results and Discussions:** Assay adaptations such as wash steps, using different procedures for the same assay, using alternative reagents, are providing better results that ensures the endpoints measured are due to genuine effects of NPs on the cells and minimise NPs-interactions with the assays. **Conclusion:** *In vitro* cell cultures-based assays require optimization to overcome major challenges with assay interference when it comes to *in vitro* NP toxicology testing.