Replacement of the *in vivo* Bioassay for Erythropoietin with the *in vitro* Bioassay

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In vivo bioassays for biological medicines have long been considered final resort to unequivocally assess the biological activities for them because there are some cases in which the biological activities obtained from *in vivo* bioassay and *in vitro* bioassay quite differ each other. Erythropoietin (EPO, epoetin) is a wellknown example among these. However, there has also been strong request to replace *in vivo* bioassays with *in vitro* bioassays or other physicochemical means in that *in vivo* bioassays give rise to many cumbersome problems such as high cost for the animals used, laborious procedures, significant fluctuation of data points leading to uncertainty of test results, and ethical matters involved in animal usage. EPO has been well characterized as to its biochemstry and structure-function relationship and its manufacturing process is known to be stringently controlled for uniform production in many pharmaceutical companies even in Korea. Therefore, EPO could be a good candidate for the project which deals with replacement of *in vivo* bioassay. The *in vivo* biological activity of EPO depends on its sialic acid contents which confer microheterogeneity-isoforms to this protein. EPO isoforms with low sialic acid contents have lower *in vivo* biological activity due to fast clearance in blood circulation. These isoforms can be detectable by isoelectric focusing technique. We have devise a method which consists of a *in vitro* bioassay using BaF3 cell line and a capillary zone electrophoresis (CZE) for the measurement of the EPO isoform distribution. The isoform distribution for EPO-BRP (European Pharmacopoeia) by CZE method resulted in isoform 2 through isoform 8. The major peaks in electrophoregram were composed of isoform 3 through 7. Our recombinant EPO having equivalent *in vivo* biological activity showed the isoform distribution of isoform 3 through 9. The biological activity of EPO obtained using *in vitro* bioassay with BaF3 cell line showed good correlation to EPO content measured by either spectrophotometric assay or radio immunoassay. The assay

validation for the *in vitro* bioassay confirms the robustness of our method in terms of precision, accuracy, repeatability.

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