Reply to: [Comment to: Recombinant erythropoietin found in seized blood bags from sportsmen. Haematologica 2008;93:313-4

We appreciate the comments from Lippi *et al.* on our previous letter[1] addressing the detection of rHuEPO in seized plasma bags and its potential further usefulness. We are pleased to forward some relevant clarifications. The availability of blood (plasma or serum) as a biological matrix for testing of doping agents in sport will undoubtedly increase in the immediate future. Not only the seize of blood bags as per our previous letter but the request of blood testing for human growth hormone, blood transfusions, synthetic haemoglobins, insulin or the establishment of the athletes blood "passport" by the World Antidoping Agency and major international sport federations will largely increase the availability of blood (often without urine) in dope testing. As recognized by other comments of the same mentioned authors,[2] the storage of athletes blood samples represents a preventive strategy, allowing retesting once a definitive test - as the one proposed in our letter - be available. In all such cases, evidence of rHuEPO isoform profile in blood will be the unequivocal proof of doping.

Subcutaneous (sc) injection is the usual route of rHuEPO administration in athletes.[3] It is not surprising as this route had been claimed more effective than intravenous application.[4] Obviously, basic pharmacokinetics indicates that, for any substance, the time detection window in plasma will be shorter than in urine. However, the studies carried out in athletes,[5] even from different ethnic groups,[6] confirm that beyond 24 hours after last sc rHuEPO administration, substantially high mean plasma concentrations (higher than 20 IU/L) are still found. Those values are fully compatible with our proposed methodology and therefore any exogenous EPO present in plasma will be easily recognized. In other aspects regarding new EPO analogues or mimetics, contrarily to what is mentioned in Lippi et al letter, Dynepo and CERA are detectable[7,8] and, in particular CERA has a limited excretion in urine. Immunopurification previous to isoelectrofocusing analysis of EPO is being now recommended even for the routine urine EPO test to warrant better limit of detection, repeatability or even simplify or speed up the sample preparation. Thus, even for urine, the use of immunopurification avoids the need of costly repetition of problematic urine samples. It is rewarding to note that Lippi et al. seem to agree with our opinion that the best opportunity to test for EPO is not in competition but out of competition in "intelligent" unannounced testing situations. Interestingly, the same rationale could be applied to the testing for anabolic agents but nobody has ever suggested stopping testing for those substances in competition. Regarding costs, in other areas of doping control the ratio testosterone to epitestosterone has been reduced from 6 to 4 as the cut-off to trigger a sophisticated and expensive confirmation of exogenous testosterone. On a similar line, many sport authorities consider that the cost-benefit of the EPO test, when considering EPO as one of the most powerful doping agents, is worth the value. The future challenges in doping control are mainly peptide hormones, precursors, or even gene manipulations. Those challenges can only be faced using sophisticated techniques which undoubtedly will not be cheap, but will be the key for a clean and fair sport. Thus, in comparison, testing for EPO in blood is not expensive. For us, EPO blood analysis is also practical, affording suitable complementary tools to the present anti doping potentialities.

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