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## **Preliminary excretion study of CERA in human urine and serums: which is the better matrix for detecting CERA abuse?**

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### *Abstract*

This page presents a result of Continuous erythropoietin receptor activator (CERA) analysis in both urine and serum with the detecting method used by doping control laboratories. Samples were collected from two Asian volunteers after a single dose of CERA injection. Considered the discontinuous renal excretion shown in the results, serum test is a more efficient and reliable way to detect CERA.

### *1. Introduction*

Continuous erythropoietin receptor activator (CERA) is the third generation of erythropoiesis stimulating agents (ESA), which has been reported to be misused in endurance sports in recent years, several studies have been carried out to improve detection of CERA for doping control. Compared with other ESAs, CERA differs in both blood distribution and renal excretion.<sup>[1, 2]</sup> This page describes the preliminary results of CERA analysis in urine and serum samples, collected from two volunteers after a single subcutaneous injection (50µg/0.3mL), using isoelectric focusing (IEF) and Western Blotting method together with the MicroCoat® MIRCERA ELISA kits.

### *2. Experimental*

A single dose of Mircera® (50µg/0.3mL) was administered subcutaneously to 2 volunteers (information listed in Table. 1). All the urine was collected for the first 3 days and morning

urine only was collected for the following 7 days, serum samples were collected 1 hour before the injection and 1, 2, 3, 4, 6, 8, 10, 13, 16, 20, 24 and 50 days post-injection.

Table 1. Information of the volunteers.

	Gender	Age	Body Weight
Volunteer 1	Male	42	66kg
Volunteer 2	Female	27	51kg

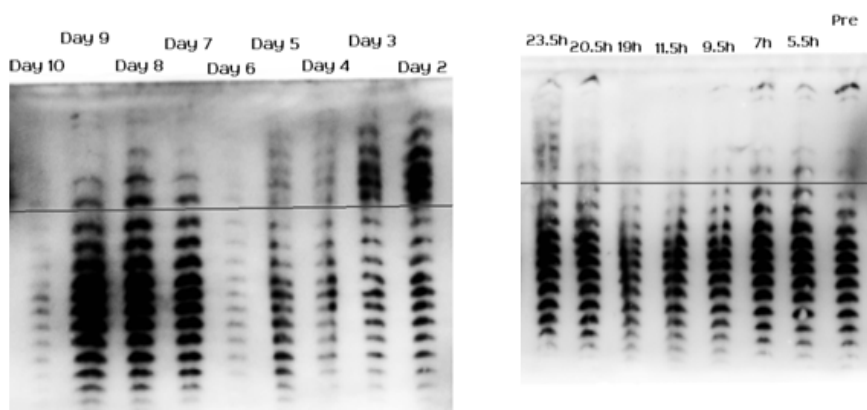
All urine samples were prepared, applied to IEF (pH 2-6) and determined using the published double blotting method<sup>[3]</sup>. Serum samples were analyzed with the Mircera® ELISA kit manufactured by MicroCoat Biotechnologie© and Roche Diagnostics©, following the instruction manuals<sup>[4]</sup>. Serum samples were also purified using an immunoaffinity column<sup>[5]</sup> and the IEF plus double blotting procedure.

Mircera® Syringes were kindly provided by Dr. Christian Reichel from ARC, doping control laboratory.

### 3. Results and discussions

#### 3.1 IEF results of urine samples

Fig. 1, CERA profile in urine of volunteer 1.

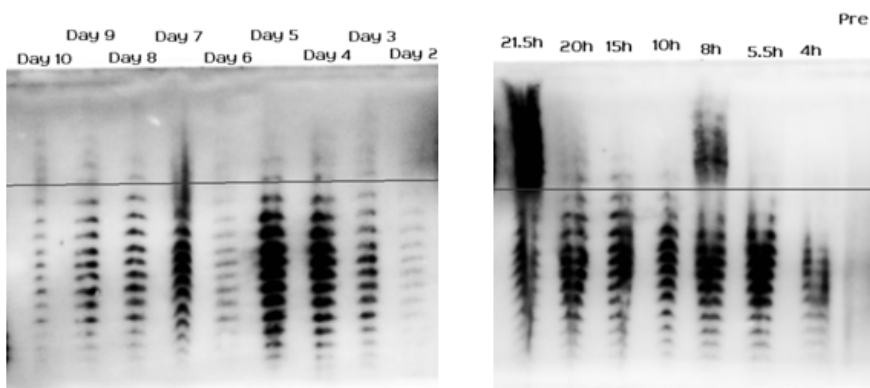


For the male volunteer, CERA bands could not be detected within 23 hours post administration, and then were clearly detected for the first 5 days, slightly decreasing on days 4 and 5. From day 6 to day 8, there were still bands appearing in the CERA area, but these were not sufficient for fulfilling the WADA criteria of positivity (Fig. 1).

For the female volunteer, clear CERA bands were observed at erratic times post administration (8h, 21.5h), then some faint CERA bands showed from day 2 to 5, some bands were almost invisible from day 6 to 10. The profile of the 8-hour-urine was smeared in the target area, which may have been caused by an extremely high concentration of CERA in this

sample, but the EPO concentration shown by the DPC immunoassay was similar to that of the others. This indicated a discontinuous renal excretion of CERA (Fig. 2).

Fig. 2, CERA profile in urine of volunteer 2.

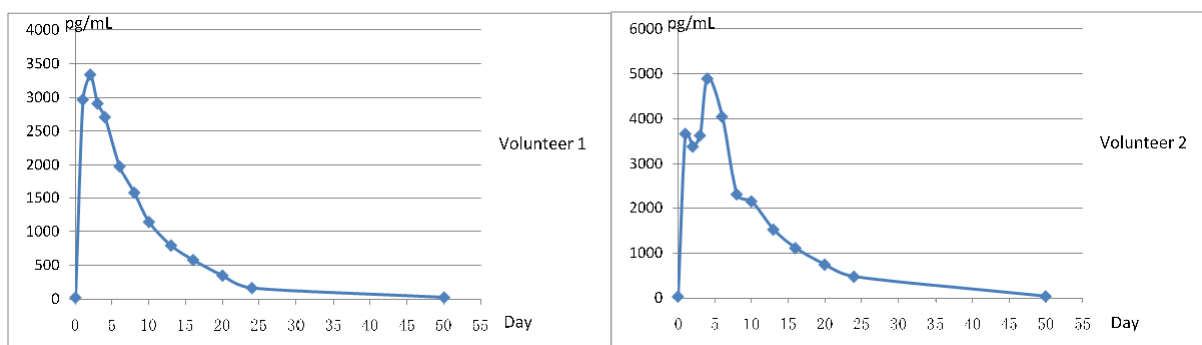


### 3.2 ELISA and IEF Results of Serum sample Tests

Some of the serum samples were diluted 5, 10 and 20 times to meet the quantification range of the ELISA assay using the calibration curve of  $y=1.14x+2.73$ ,  $R^2=0.9985$ .

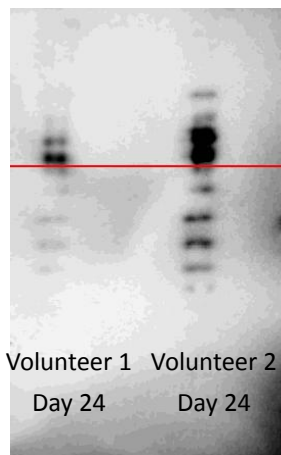
In the results listed in Fig.3, both of the volunteers' serum CERA levels were significantly raised on the first day after injection and peaked on day 5. The protocol was originally designed to collect serum for only 24 days, but the concentration of CERA was still higher than the criteria of  $100\text{pg/mL}^{[4]}$ , therefore another sample was collected on day 50.

Fig. 3, CERA serum concentration- Time Curve



The serum collected on day 24, which was with the lowest concentration above  $100\text{pg/mL}$ , was applied to an IAC purification and IEF plus double-blotting assays. Lanes of these two samples were much fainter than the standards used in our routine procedure ( $1500\text{IU/L}$  of EPO), the absolute pixel volume determined by GASepo software was around 10 times less than the standards (Fig. 4).

Fig.4, IEF profiles of serum collected on day 24



#### 4. Conclusion

After a single dose of 50µg Mircera®, CERA could only be detected in urine from 24 hours to 5 days, and not all the samples could be clearly judged as CERA positive; the blood concentration stayed above 100 pg/mL for at least 24 days for both volunteers. Regarding the IEF profiles of the serum with the lowest CERA concentration, serum ELISA test followed by IEF confirmation appeared to be a more efficient way to detect CERA abuse.

#### Acknowledgements

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#### References

- [1] Provenzano R, Besarab A, Macdougall IC. (2007) The Continuous Erythropoietin Receptor Activator (CERA) Corrects Anemia at Extended Administration Intervals in Patients with Chronic Kidney Disease Not on Dialysis: Results of a Phase II Study. *Clin Nephrol* 67(5), 306-317.
- [2] McGahan L. (2008) Continuous Erythropoietin Receptor Activator (Mircera) for Renal Anemia. *Issues Emerg Health Technol* 113, 1-6.
- [3] Lasne F, Martin L, Crepin N, de Ceaurriz J. (2002) Detection of Isoelectric Profiles of Erythropoietin in Urine: Differentiation of Natural and Administered Recombinant Hormones. *Anal Biochem* 311, 119-126.
- [4] Lamon S, Giraud S, Egli L, Smolander J, Jarsch M, Stubenrauch K, Hellwig A, Saugy M, Robinson N. (2009) A high-throughput test to detect CERA doping in blood. *J Pharm Biomed Anal* 50, 954-958.
- [5] Lasne F, Martin L, Martin JA, de Ceaurriz J. (2007) Isoelectric profiles of human erythropoietin are different in serum and urine. *Int J Bio Macromol*, 41(3), 354-357.