A pre-analytical challenge to determine estradiol in children: A monovette systematically causing increased estradiol-concentrations in LC-MS/MS analysis

T. Lamprecht[1], M. Kleber[2], J Rothermel[2], PM Holterhus[1], and AE Kulle[1]
1. University Hospital Schleswig-Holstein, Campus Kiel, Children's Hospital, Department of Paediatrics, Division of Pediatric Endocrinology and Diabetes, Christian-Albrechts-University of Kiel
2. MVZ Katholisches Klinikum gGmbH, children's hospital, department for children's endocrinology and diabetology, Bochum, Germany

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INTRODUCTION

In children, 17-β-estradiol (E2) concentrations are 1000-times lower in comparison to their precursors, the androgens. Depending on gender, age and pathology plasma concentrations vary in a broad range. The sensitive and specific determination of E2 is a particular challenge in the endocrine analysis.

- female patient
- 12 6/12 years, Tanner stage B1
- presumed diagnosis: ovarian insufficiency
- high E2 concentration of 296 pmol/L (age reference 10-430 pmol/L, 12-14 years) does not suit with the clinical diagnosis and Tanner B1

A different blood collection tube was used than typically preferred by us.

AIM

- The aim was to identify possible interfering factors by blood collection tubes.

METHOD

- E2 was determined by LC-MS/MS.
- **A:** Two new blood samples of the patient were collected independently in two different types of tubes. One tube was the same used beforehand (Kabe Labortechnik) and the other one typically preferred by our pediatric laboratory (Sarstedt).
- **B:** Tubes were broken down into their components (gel, pellets, and hole tube). The components were incubated independently with either aqua dest. or strip.-plasma for one week.

RESULTS

A

- On average ten times higher E2 concentration was determined in the patient using the Kabe tube than using the tube produced by Sarstedt (Table 1).
- An unusual broad peak was noticed at the typical position of the E2 peak using the Kabe tubes (Fig. 1 & 2).

Device	Kabe	Sarstedt
Repetition	3	5
E2 average [pmol/L]	147,947	15,133

Table 1: mean E2 concentration of the female patient differing between the two tubes.

B

- After incubating each component of the tubes, the gel chromatogram of the Kabe tubes showed a similar wide peak (Fig. 3).
- Plasma that was incubated with the gel of the Kabe tube shows a wide peak that leads to a high E2 concentration (Fig. 3).
- Possible isomeric interference could be excluded by chromatographic separation of 17-α and 17-β-E2 into two separated peaks (Fig 4).

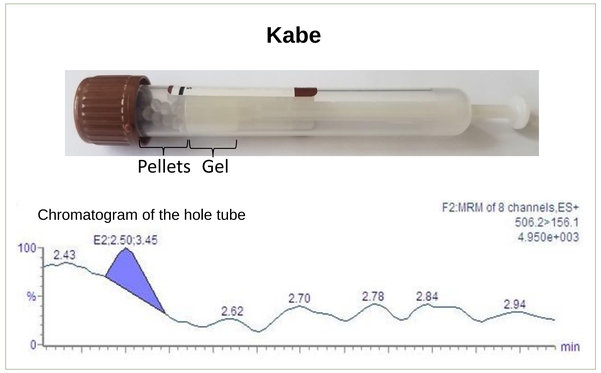

Figure 1: Kabe tube with its two components and the corresponding original chromatogram (y-axis: relative intensity; x-axis: retention time) with a great E2 peak at the typical position.

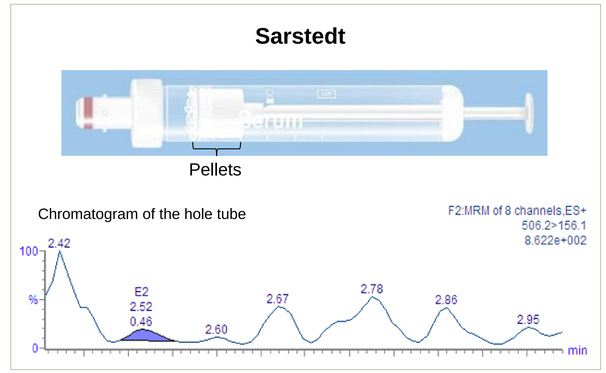

Figure 2: Sarstedt tube with its pellets components (no gel) and the corresponding original chromatogram (y-axis: relative intensity; x-axis: retention time), showing a small E2 peak at the typical position.

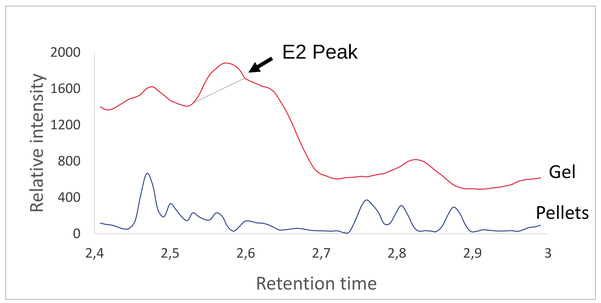

Figure 3: Chromatogram with the relative intensity on the y-axis and the retention time on the x-axis made with excel, showing the comparison of the chromatograms after incubation plasma with the Kabe gel and the pellets.

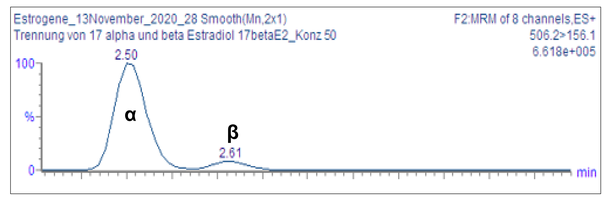

Figure 4: Original chromatogram with the relative intensity on the y-axis and the retention time on the x-axis made with excel, showing the separation of 17-α- and -β-estradiol.

CONCLUSIONS

- The majority laboratory errors (~46–68.2%) originate in the preanalytical phase
- Typical pre-analytical interfering factors are lipemia and haemolysis
- Blood collection tubes are an often under-recognized variable in the preanalytical phase of clinical laboratory (Bowen et al 2016)
- Especially in analysis of E2 which require greater analytical sensitivities
- Paediatric hormonal measurements should involve pediatric endocrine validation to notice discrepancies
- Therefore, automated evaluation should not be used for E2 determination in LC-MS/MS.

REFERENCES

1. Bowen RAR, Adcock DM. Blood collection tubes as medical devices: The potential to affect assays and proposed verification and validation processes for the clinical laboratory. Clin Biochem. 2016 A.D.; 49:1321–1330. doi: 10.1016/j.clinbiochem.2016.10.004. Cited in: PMID: 27765677.

CONTACT INFORMATION

ePoster Code: P1-147
Session Name: Sex Endocrinology and Gonads B
email: Tabea.lamprecht@uksh.de

UKSH UNIVERSITÄTSKLINIKUM Schleswig-Holstein