



## Letter to the editor



## Daisy-like crystals: Not just the result of sampling artifact and not only in urine

In a recent issue of this journal, Hudák and co-workers reported on daisy-like crystals (DLcr) found in the urine of a 5-year-old female child, collected in a glass jar which had been sterilized by using tap water boiled for 15 min and left to cool overnight [1]. The same crystals were also found in two samples containing only hard water, again boiled and then cooled: one collected by the patient's family at laboratory request, the other collected under experimental conditions in the laboratory. These findings led the authors to hypothesize that DLcr previously described by us [2,3] and by Frochot and co-workers [4] could be the result of urine contamination due to urine collected in containers sterilized at home by using boiled water with a high limescale content.

In order to verify the hypothesis proposed by Hudák and co-workers, we have retraced the urine collection procedures used by the subjects described so far [2–4] by contacting the laboratory professionals who had found DLcr in their urine. The results, reported in Table 1, demonstrates that 9 out of 11 subjects had used appropriate sterile plastic containers (tubes or jars), either bought in pharmacy or supplied by laboratories, while for 2 other subjects no information could be retrieved (Table 1: patient 4 did not remember how she had collected the urine in October 2011; patient 8, a 93-year-old woman in 2016, could not be reached).

Table 1

The container used for urine collection by the patients described in Refs. [2] (\*), [3] (\*\*), and [4] (§).

Subject	Gender/Age (years)	Date/Country	Source	Urine collection
1*	Female/30	March 2003/Italy	Silvia Baroni	Sterile plastic jar
2**	Female/40	August 2006/Croatia	Dunja Rogic	Sterile plastic tube
3**	Female/3	April 2003/Italy	Carla Covarelli	Sterile plastic tube
4**	Female/38	October 2011/Italy	Roberta Anderlini	Not available
5**	Male/19	March 2014/Belgium	Jérémie Gras	Sterile plastic jar
6**	Female/77	February 2015/Italy	Roberta Anderlini	Sterile plastic tube
7**	Female/78	September 2015/France	Alexandre Teboul	Sterile plastic tube
8**	Female/93	May 2016/Italy	Roberta Anderlini	Not available
9**	Female 47	December 2016/Italy	Antonella Proietti	Sterile plastic tube
10**	Female/82	January 2017/Italy	Simone Canovi	Sterile plastic tube
11§	Female/64	July 2016/Belgium	Vincent Castiglione	Sterile plastic jar

As to the urine of the dog containing DLcr [3], we now know from one of us (J.K.) that it had been collected in a non-sterile plastic container, which had been cleaned with warm water and soap [5]. Thus, for that case we cannot exclude that DLcr might have been caused by urine contamination from water. However, after that case, small amounts of DLcr have been found in the urine of two other dogs, in one of which urine had been obtained by direct bladder puncture and in the other by bladder catheter. In both cases the urine had been collected in a sterile plastic syringe and examined with manual microscopy [5].

Last but not least, DLcr have been found by one of us (S.B.) in the seminal fluid of two male patients who had been investigated for infertility. For both patients the sperm had been collected, after few days of sexual abstinence, in a plastic sterile jar and was examined by manual microscopy at 400× magnification one hour after ejaculation [6]. Patient 1 was a 21-year old man, whose sperm, examined on February 10th 2010, contained 9 DLcr/10 µL; patient 2 was a 37-year-old man whose sperm, examined on December 12th, 2019, contained 4 DLcr/10 µL.

In conclusion, if on one hand the paper of Hudák and co-workers demonstrate that DLcr in the urine can be due to a sampling artifact, on the other our data clearly demonstrate that other factors, not associated with urine collection procedures, can favour the precipitation of DLcr in urine. All this highlights the importance of using proper sterilized urine containers. Finally, we now know that DLcr can also be found in seminal fluid, besides in the parotid saliva of dogs as reported by Charles-Philippe Robin and François Verdeil in 1853 [7].

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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