The EBF DBS Microsampling Consortium: Stability sub team

Age of Control (Wet) Blood

Background and Aim

There are still uncertainties about how long control blood may be stored before use in DBS. In the past the blood has been stored up to 14 days.
The current work was done to investigate the effects of control blood age, for different species, anti-coagulant and substrates on the size of the dried blood spots with a view to determining how long the blood can be stored. In addition, physical observations of the control blood were recorded.

Team Members

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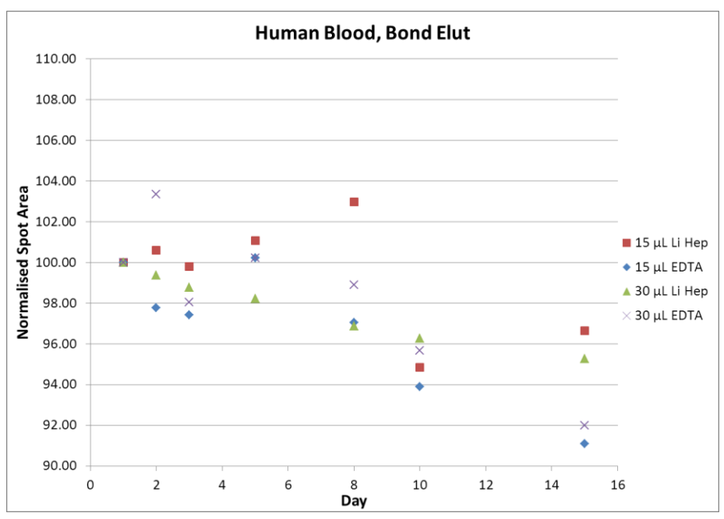
Ongoing Activities and Current Results

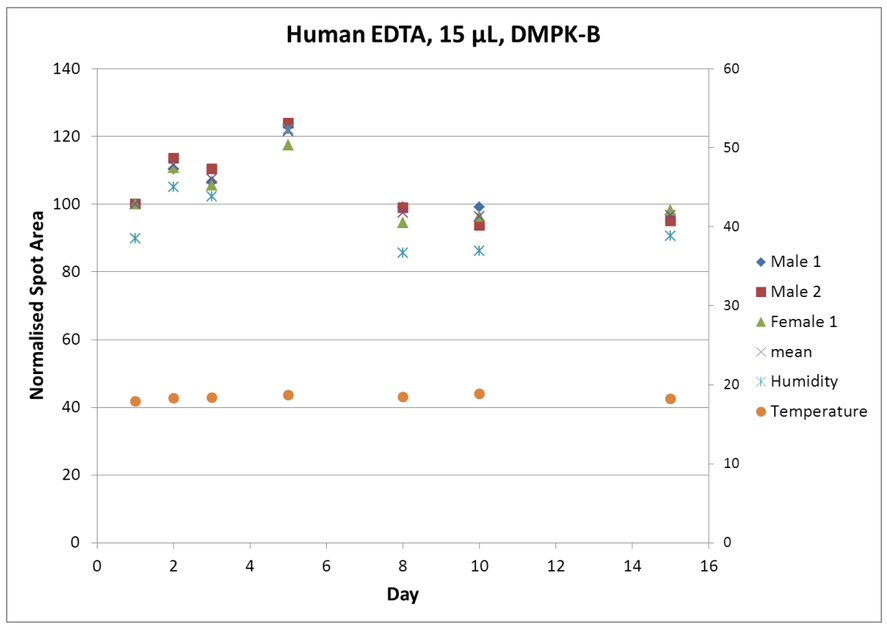
❑ Method

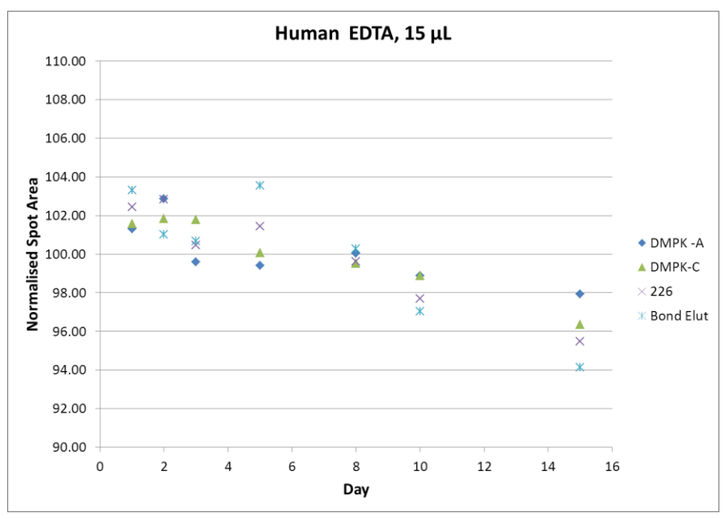
- Four spots were applied on a DBS card for each of the tested conditions
- Cards were air dried for 2 hours and a photo taken
- Areas of the spots were calculated using Image J
- Temperature and humidity conditions were monitored during the test.
- Physical observations pertaining to the condition of the blood were also recorded
- Blood was stored at 4°C whilst not in use

Variables were as follows:

- Control blood: human, dog, rat and mice
- Anticoagulants: Li-hep and EDTA
- Day of spotting: Day 1 (day of collection), 2, 3, 5, 8, 10 and 15.
- Card: DMPK-A, -B and -C, 226, and Bond Elut.
- Spot volume: 15 and 30 µL
- Comparison of live bled animals and CO_2 euthanised animals in rat only.







❑ Results

All spot size data over 15 days was normalised to the average for that card type, anti-coagulant and species. Although spot sizes were generally stable with age of wet blood, a few observations could made;

- For rats, the spots produced from live bleed samples were smaller on day 15, an observation which was not true for the blood taken from CO_2 euthanised animals and is probably related to the reported "crusty" nature of the spots

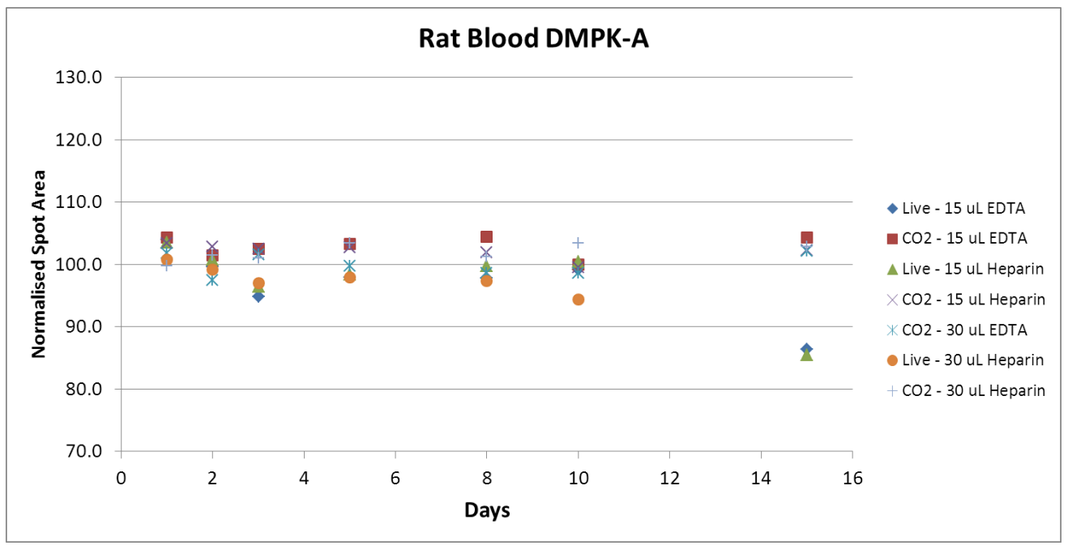
For human blood:

- spot size correlated to changes in humidity on DMPK-B cards
- measured areas diminished over time on Bond Elut cards
- human EDTA blood spots diminished in size upon storage of the blood
- For both mouse and dog blood there were no observable trends in spot areas over time
- Larger intra- and inter-day variability was noted using the Bond Elut type cards

❑ Physical Observations

Most observations were related to the changes in characteristics of the wet blood samples over time.

- The older the blood, the longer it takes to absorb into the cards (rat EDTA at 15 days being the extreme example)
- The blood becomes more viscous from day 5 onward
- From day 5 onward there is formation of blood clots.
- Li-hep blood tends to stick to the walls of the tubes more than EDTA blood
- Bond Elut cards were noted as being prone to spiky spots resulting in greater variability upon size measurement
- Spot sizes differed between cards with spots tending to merge when spiking 30 µL onto DMPK-B cards



Future Plans

- ❑ Publish a scientific paper summarising the age of blood experimental data containing conclusions about storage time of blood matrices in function of species, anticoagulant and card type.
- ❑ Publish a scientific paper on spot homogeneity as a function of age of blood using radioactive compounds.

The data presented herein are derived from investigations at EBF consortium member laboratories in line with pre-defined experimental criteria. It is not the intent of the EBF DBS Microsampling Consortium to endorse any specific product above others. Any limitations observed with certain products are a reflection of the specific experiments conducted for the test materials under investigation.