Automated mouse tail tattoing identification method: an evaluation at iCS

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Background

- iCS Mice for phenotyping are identified by toe tattooing:
  - Works in practice
  - Need to be well trained
  - Remarking is sometimes necesary
  - Handling of animal to read identification
  - Transcription gap or misidentification may happen

- The Need :
  - To find a more *reliable*, and *straightforward* way to ID mice
  - Identification method must be *compatible* for phenotyping testing
  - To have a visual ID (key for mice handled by many people during phenotyping process

iCS Evaluation of an Automated mouse tail tattooing identification method

- The automated mouse tail tattooing Labstamp System®

LABSTAMP SYSTEM:

1 Applicator Machine

2 Restraint Cartridges (SMALL and/or LARGE)

3 Tail Gauge

4 Needle Cartridges (Yellow and Green)

5 Ink Slides (Black or Green, in foil pouches)

6 Tail Oil

iCS Evaluation of an Automated mouse tail tattooing identification method

- Automated mouse tail tattooing Labstamp System® Demo:

Study Plan

- To evaluate practically this automated Tattoo system:
  - C57BL/6N males mice at different age:
    - What is the youngest age for tattooing ?
    - Difference in tattoo localisation with the age of identification ?
  - Average time needed per mouse
  - Tracking / Monitoring of the tattoo readibility and durability

- Compatibility with some tail-based phenotyping test
  - Non-Invasive blood pressure and Heart rate by the tail-cuff method
  - Pain sensitivity measurement (Tail Flick test)
  - Motor ability measurement (EMG test)

Study Plan

- 1 cohort / age, 10 M B6N tattooed/Cohort

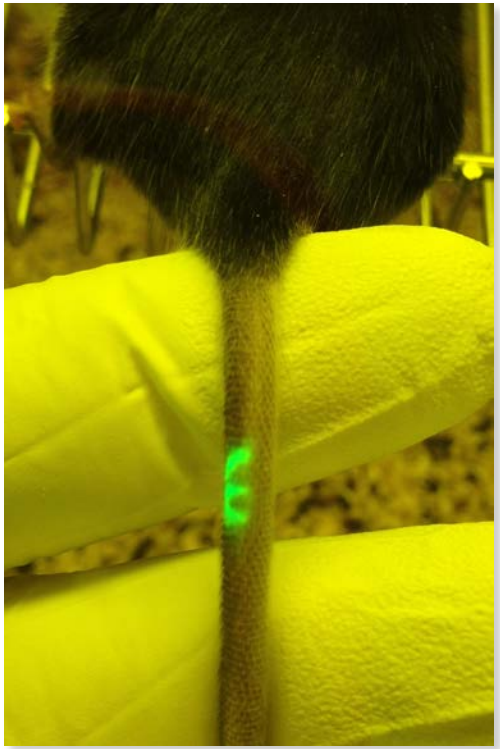
Cohorte Series	Age of tattooing	IDs	Phenotyping	Start of Phenotyping	End of phenotyping
300	3 weeks	301-310	yes	33/2012	43/2012
400	4 weeks	401-410	no	N/A	N/A
500	5 weeks	501-510	yes	29/2012	39/2012
700	7 weeks	701-710	yes	30/2012	40/2012

- Tracking of Ids Readibility

RATING	Definition
-	Unreadable
+/-	Ambiguous
+	Readable
++	Clear
+++	Very Clear

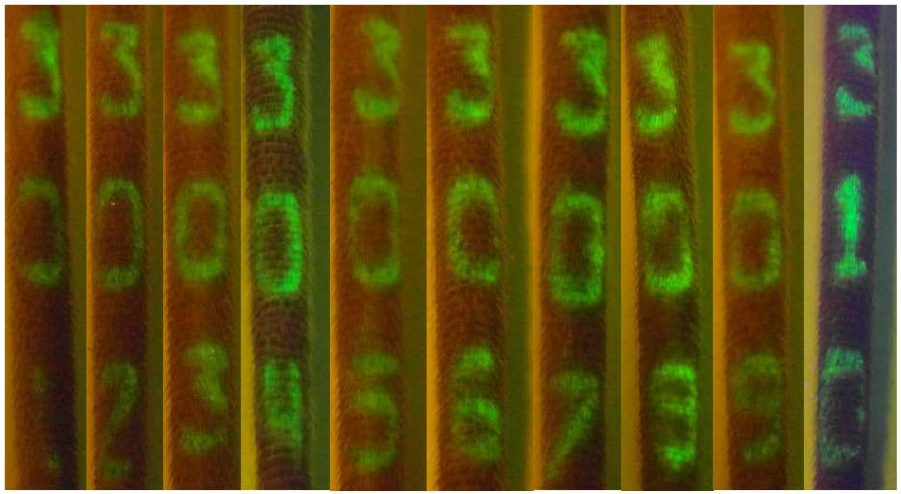
ink tattoo

Green ink suggested for C57BL/6 mice



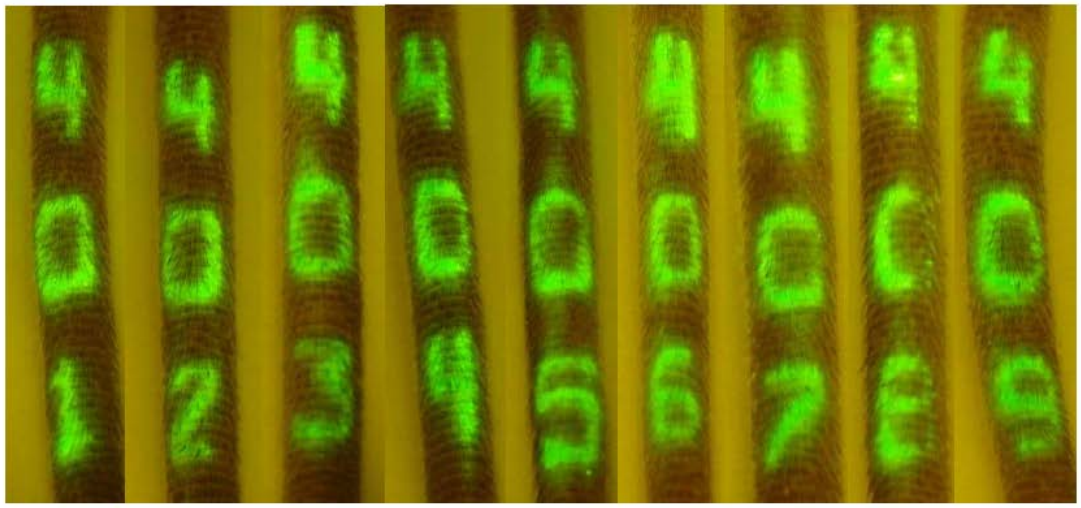
Tattooing at 3 weeks

ID & DOB 25/12	Cartridge Needle	28/12	29/12	30/12	31/12	32/12	34/12	36/12	38/12	42/12	06/13	33/13
301	SY	+	+	+	+	+	+/-	+/-	+/-	+/-	+/-	+
302	SY	+++	++	++	+++	+++	+++	+++	+++	+++	+++	++
303	SG	+++	++	++	+++	++	++	++	++	+++	++	++
304	SG	+++	++	++	+++	++	+++	+++	+++	+++	+++	++
305	SG	+++	+++	++	+++	+++	++	++	++	+++	+++	+++
306	SG	++	++	++	++	++	++	++	++	+++	+++	+++
307	SG	+++	++	+	++	+++	++	++	++	+++	++	+++
308	SG	++	+	++	++	+	+	+	+	+	+	+/-
309	SG	+++	++	++	++	++	++	++	++	++	++	+++
310	SY	+++	+++	+	++	++	++	++	++	++	++	+++



Tattooing at 4 weeks

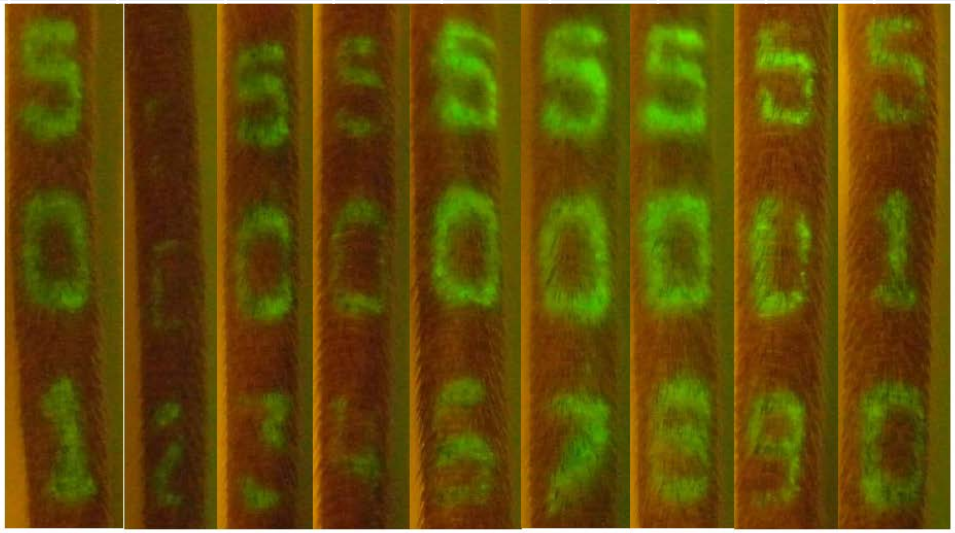
ID & DOB 22/12	Cartridge Needle	26/12	27/12	28/12	29/12	30/12	32/12	34/12	36/12	38/12	42/12	06/13	33/13
401	SG	++	++	++	++	++	++	+	+	+	+	+	+++
402	SG	+	+	+	+	++	+	+	++	++	+	+	++
403	SG	+	+	+	+	+	+	+	+	+	++	++	+++
404	SG	+	+	+	+	++	+	++	+	+	++	++	++
405	SG	+	+	++	+	++	+	++	++	++	++	++	+++
406	SG	+	+	++	++	++	+++	++	++	++	+++	N/A	N/A
407	SG	+	++	+++	+++	++	+++	+++	++	++	+++	+++	++
408	SG	+	+	+	+	++	++	++	++	+++	++	++	+
409	SG	+	+++	++	++	++	++	+++	+++	+++	+++	+++	+++
410	SG	+	+++	+++	+++	++	++	++	+++	+++	+++	+++	++



N/A= deceased

Tattooing at 5 weeks

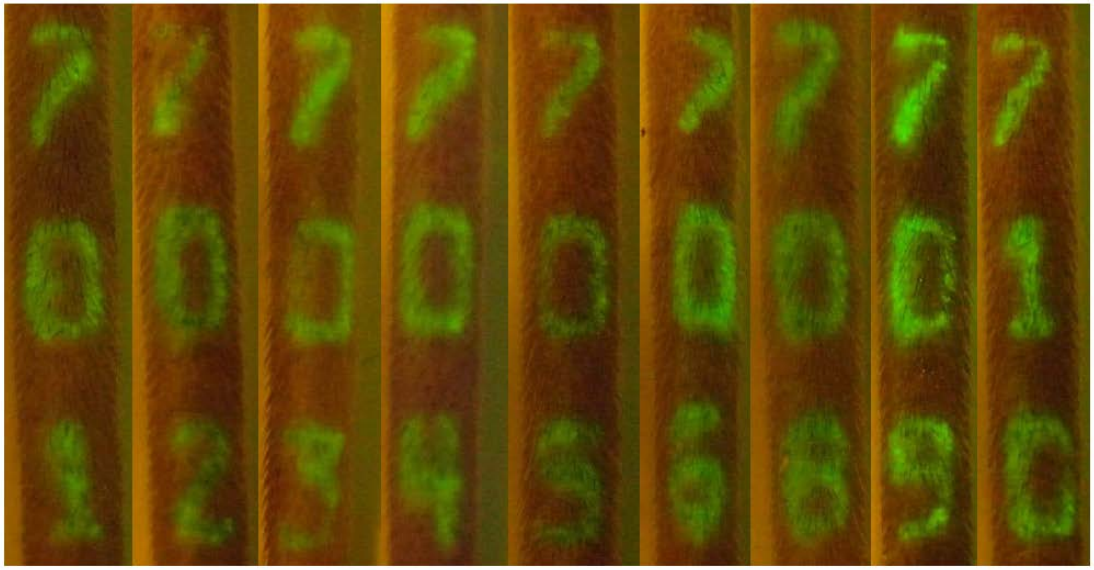
ID & DOB 21/12	Cartridge Needle	26/12	27/12	28/12	32/12	33/12	36/12	38/12	42/12	02/13	33/13
501	LY	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
502	LY	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-	+/-
503	LY	++	++	++	++	++	++	++	++	++	++
504	SG	++	++	++	+++	+++	+++	+++	+++	+++	+++
505	SG	+++	+++	++	+++	+++	+++	+++	+++	N/A	N/A
506	SG	+	+	++	+++	+++	+++	++	+++	N/A	N/A
507	SG	++	++	++	+++	+++	+++	+++	+++	+++	+++
508	SG	+	+	+	+++	+++	+++	+++	+++	+++	+++
509	LY	+	+	++	+++	+++	+++	++	+++	+++	+++
510	LY	+	+	++	+++	+++	+++	+++	+++	+++	+++



N/A= deceased

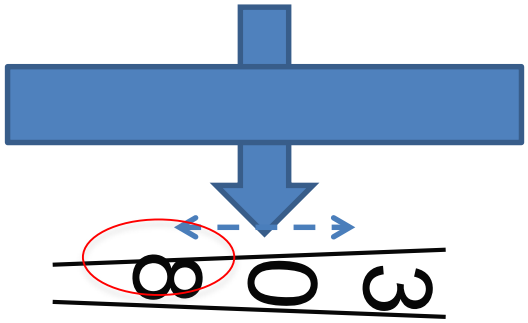
Tattooing at 7 weeks

ID & DOB 22/12	Cartridge Needle	29/12	32/12	33/12	34/12	36/12	38/12	42/12	06/13	33/13
701	LY	+++	+++	+++	+++	+++	+++	+++	+++	+++
702	LY	+++	+++	+++	+++	+++	+++	+++	+++	+++
703	SG	++	+++	+++	+++	+++	+++	+++	+++	+++
704	SG	+++	+++	+++	+++	+++	+++	+++	+++	+++
705	LY	+++	+++	+++	+++	+++	+++	+++	+++	+++
706	LY	++	+++	+++	+++	+++	+++	+++	+++	+++
707	LY	+++	+++	+++	+++	+++	+++	+++	+++	+++
708	LY	++	+++	+++	+++	+++	+++	+++	+++	+++
709	LY	++	+++	+++	+++	+++	+++	+++	+++	+++
710	LY	++	+++	+++	+++	+++	+++	+++	+++	+++



Summary I

- Age of tattooing: the older is better
  - Minimum age of tattooing : 3wks
    - Some digits may not be homogenous
    - More ambiguous Ids (last digit)
- Tail Sizing is key !
- Preference to ID mice at 4wks
- Taattoo ID :
  - is permanent
  - Is easily readable , Visual
- Average time to ID mice: 2 min
  - From tissue sampling to tail tattooing
- Users love it !



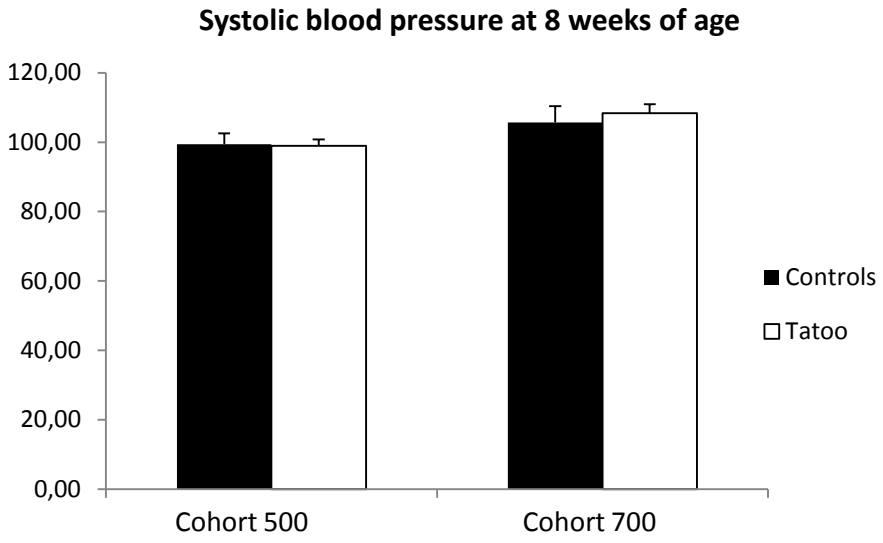
Compatibility with Phenotyping tests ?

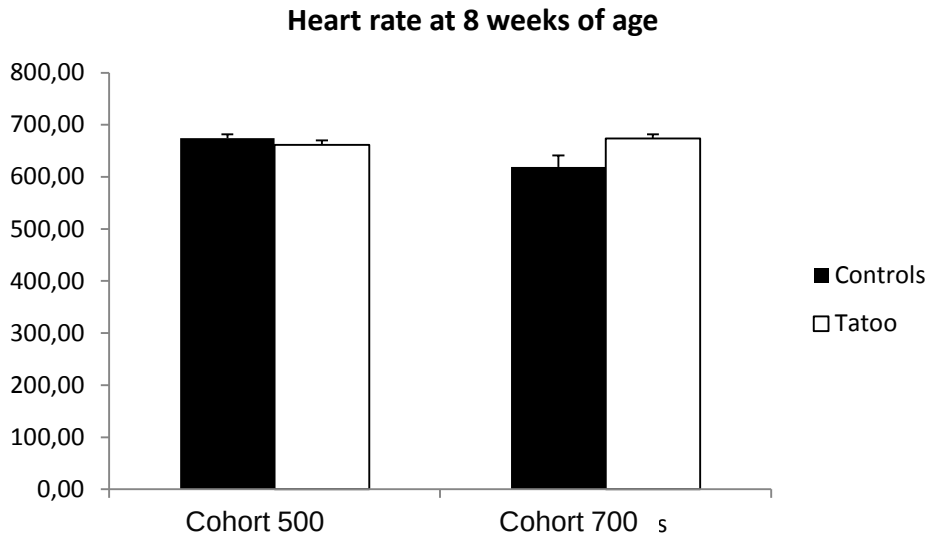
- Cohorts series :10 M B6N tattoed vs 10 M B6N littermate untattooed

Phenotyping plan	Cohort 300	Cohort 500	Cohort 700
Date of birth (week/year)	25/2012	21/2012	22/2012
Tattooed week	28/2012	26/2012	29/2012
Non Invasive Blood pressure	N/A	29/2012	30/2012
Tail Flick	42/2012	38/2012	39/2012
EMG	43/2012	39/2012	40/2012

Heart Rate and Non Invasive blood pressure

Systolic BP is measured by a computerized tail-cuff system (BP-2000, Visitech Systems, Apex, NC) in conscious animals through Photoplethysmography a light-based technology.

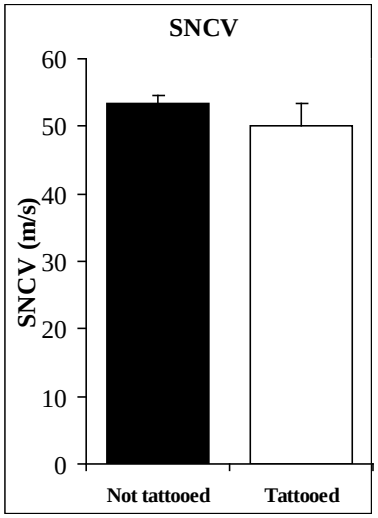




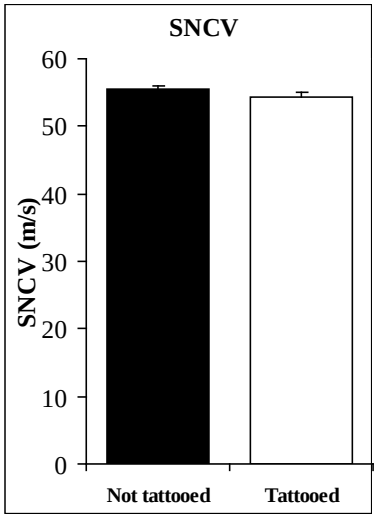
Blood pressure / Heart rate are similar

Motor ability measurement – Electromyography (EMG)

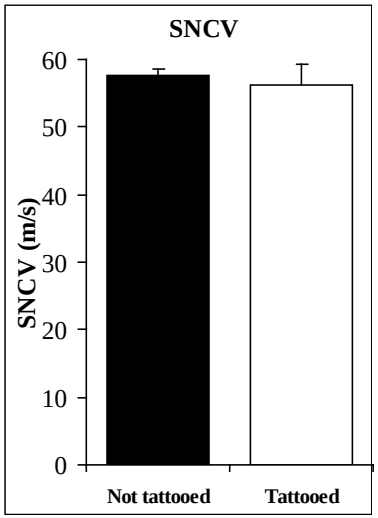
For measuring sensitive nerve conduction velocity (SNCV), recording electrodes are inserted at the base of the tail and stimulating electrodes placed 20 mm from the recording needles towards the extremity of the tail. A ground needle electrode is inserted between the stimulating and recording needles. Caudal nerve is stimulated with a series of 20 pulses during 0.2 ms each at a supramaximal intensity of 8 mA.



Cohort 300



Cohort 500



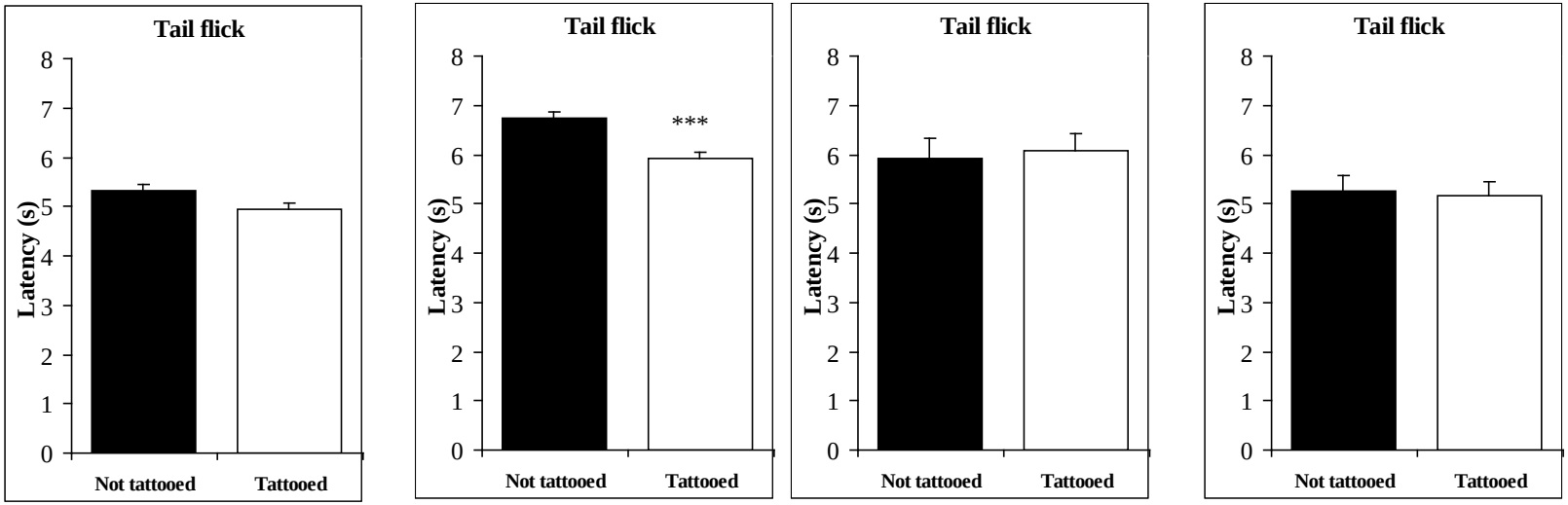
Cohort 700

Automated Tail tattoo do not alter the EMG measurment

Tail Flick: Pain sensitivity measurment

The apparatus consisted of a shutter-controlled lamp as a heat source (Bioseb). Three consecutive trials with an interval of about 1-2 min are performed at different sites of the tail.

Age at test 17 weeks 17 weeks 25 weeks 17 weeks



*** p < 0,001 - significantly different from Not tattooed

Cohort 300 Cohort 500 Cohort 700

Automated Tail tattoo seems to induce increase of pain sensitivity

Automated tail tattooing Labstamp System

- No Impact on **Non-Invasive blood pressure** and **EMG** measurment
- Tail Flick : Further studies are needed (impact of age of tattooing ?, evolution with time ?), histology)

- Black Ink is working well

- Use in routine at iCS for IMPC mice cohorts

iCS Evaluation of an Automated mouse tail tattooing identification method

PROs

- Easy, Reliable, Durable
- No Transcription gap
- Compatible with phenotyping[1]
- Identifying animals just within their cage (no handling)
- Welfare
- Ease mice distribution
- Fast adoption by Technicians

CONs

- Time to ID each animal
- Age of identification: 3 wks[2]
- Pricing: 2.7$ to 1.5$ /mouse ! !

1 : further assessment is needed for Tail Flick

2: Co-Development in progress to ID 2wks-old animals

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