

Review on the Identification Methods of Laboratory Mice, Rats, Rabbits, and Guinea pigs

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Introduction

To operate an animal laboratory efficiently it is essential to have adequate methods for the identification of individual laboratory animals. Thus, when organising the breeding program, the ability to reliably identify and group individual animals into such categories as: of similar weight, age and sex is important. Besides, also selecting and grouping animals into a program for the conduction of test(s) and research as required.

Different methods of identifying laboratory animals have been described { *Farris & Griffith Jr. (1967)*, *Lane-Petter (1967)*, *Rugh (1968)*, *Short & Woodnott (1969)*, *Hafez (1970)*, *Arington (1972)*, *Castor et. al. (1973)*, *Stonehouse (1977)*, *New (1978)*, *Harkness and Wagner et. al. (1983)*}. It was decided by the Animal Experimentation Ethics Committee (A.E.E.C.) of the National Biological Standards Laboratory (NBSL), Canberra, that there is a need to review the known methods, in order to select the most appropriate ones that also comply with the animal welfare rules and regulations. Therefore, the study described below was conducted.

The laboratory animals which are under a certain test, or kept for experimental research use must be correctly identified. Therefore, it is necessary for each laboratory animal to have it's own identity card. However, one such card may be used for a specified test on a selected animal group (as one unit) over a relatively short period of time.

The identity card may contain the following: The species of the animal, strain, date of birth, the colour of the body and eyes, and any other distinguishing features, the cage number, the method used to mark the animal, and the animal's identity number. Furthermore, it may also contain the health history and breeding re-

cord of the animal, as well as a record on the progress of the test(s) conducted, or a research project.

Methods used to mark laboratory animals

Practically, the mark should be permanent, easy to notice and identify. The methods of applying the mark should be simple, quick, and harmless to the animal. Thus, the following methods are widely used:

(1) Staining of the animals

The following stains are soluble in alcohol at 26 C. They are prepared as a 3-5% dye concentration in 70% alcohol, (with the exception being the yellow dye).

| Table 1 | |
|---------|--------------------------------------|
| Colour | Stain |
| Yellow | saturated picric acid or chrysoidin. |
| Red | acidic, basic, Carbol Fuchsin. |
| Violet | methyl violet (Gentian Violet). |
| Green | brilliant green, ethyl green. |
| Blue | Trypan blue. |
| Brown | Silver nitrate. |

A small area deeply stained is preferable to a large area lightly stained. The disadvantage of this method is that it requires re-application of the stain at regular intervals (every 2-3 weeks according to the type of stain used). Also, the stain may have side effects on the animal's coat, i.e., a loss of fur, or inflammation of the skin may occur.

On the other hand, the staining methods have many advantages and they have been routinely used in the NBSL animal breeding section, for identifying successfully different age groups in guinea pigs.

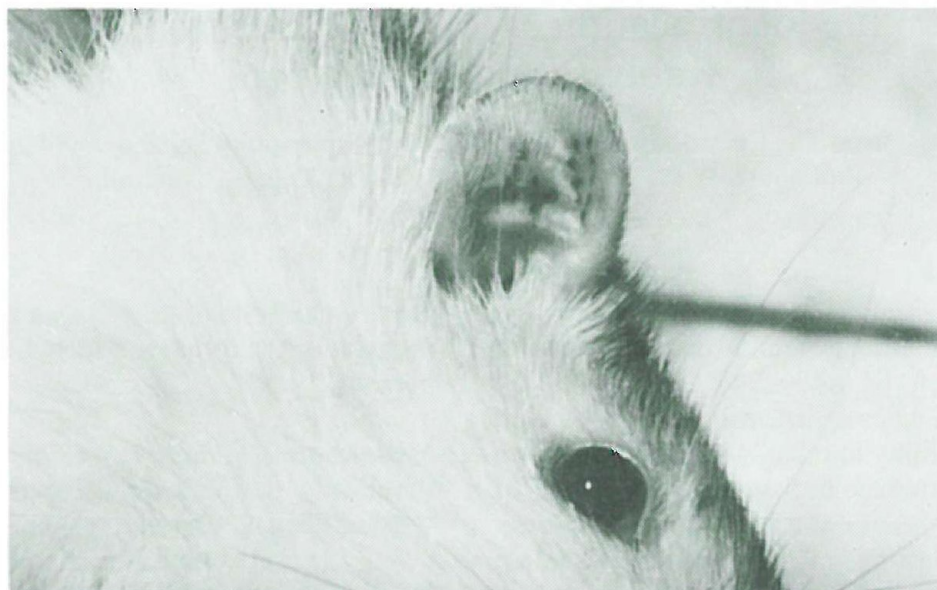


Figure 1a. Male rat (No. 40) identified by automatic tattoo pincers TZ 2L from E. Becker & Co. Ltd.

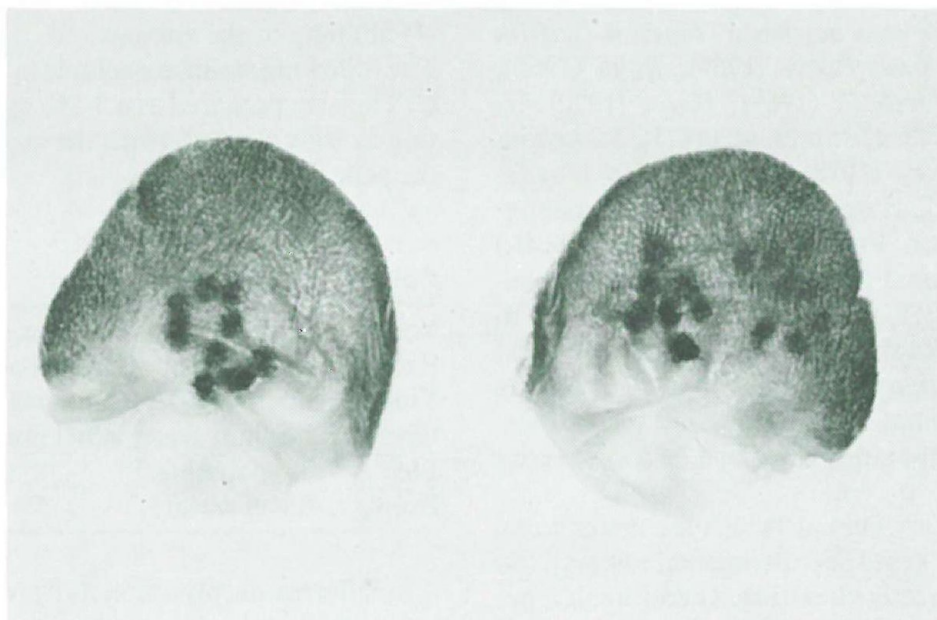


Figure 1b. Ear-tattoo in male mouse (No. 2043) that persisted for 2 years, »Knipex« tattoo pincers were used for identification at 6-weeks of age.

(2) Ear-tattooing of animals

Tattooing of animals is performed by using surgical instruments of various sizes which have a variety of interchangeable numerals and letters (Farris & Griffith Jr. 1967). The usual colour selected for non-pigmented animals is black. An electro-vibro tattoo method may also be used. The inner surface of the ear is the

usual site for the tattoo (see Figs. 1a, b).

Honma *et. al.* in 1986 described two instruments for tattooing rodents on the inner side of the ear. One of them was produced by NIPPON Boehringer Ingelheim Co. Ltd. (see Figs. 1c, d) and the other by E. Becker & Co. Ltd. (see Fig. 1e).

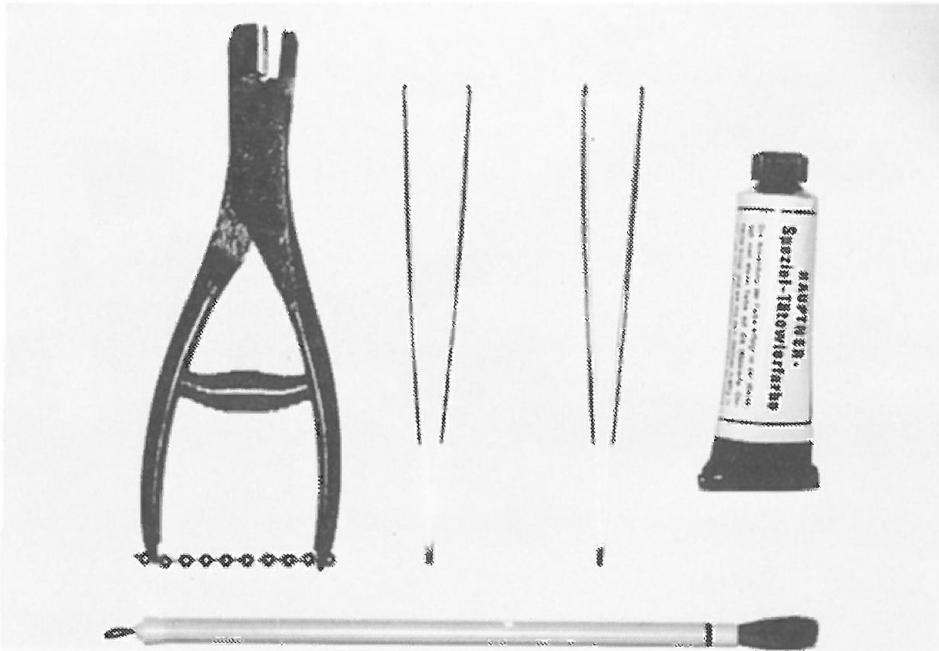


Figure 1c. The equipment needed for ear-tattooing: nut tongs »Knipex«, two pincettes, Hauptner special tattooing ink and a brush for painting.

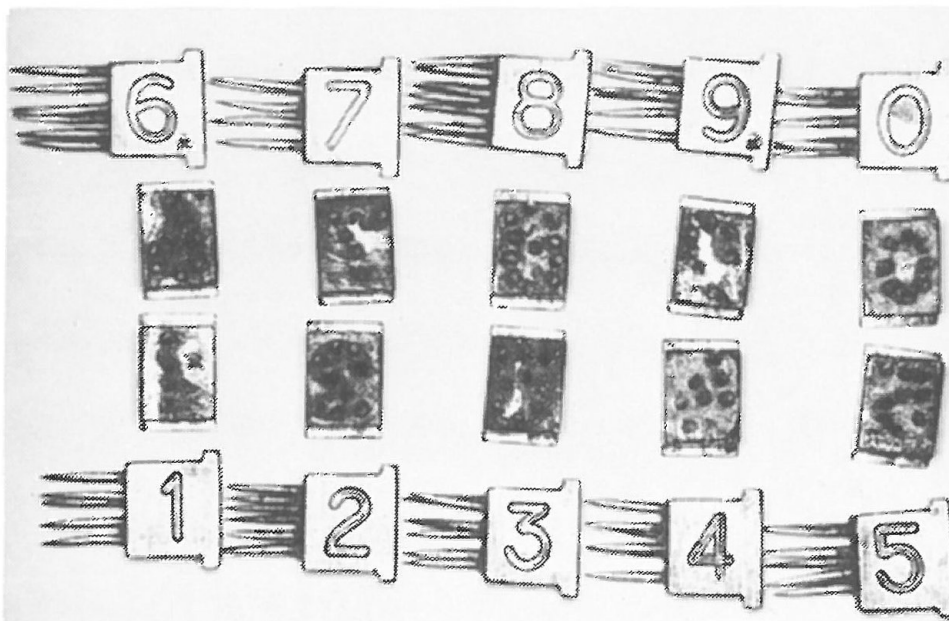


Figure 1d. A set of exchangeable needles of the »Knipex« set numbered from 0 through to 9.

Overall, this method is independent of the number of animals to be marked for an experiment, and, the duration of the experiment. Thus, it has been successfully used in acute toxicity testing, as well as, carcinogenicity studies in rodents. This method does not cause any dis-

comfort to the animal, and, it may be comparable to the technique used for performing a tattoo on people. The resulting pigmentation of the animal's ear becomes readily identifiable to the technicians.

Ear-tattooing had been used by the National

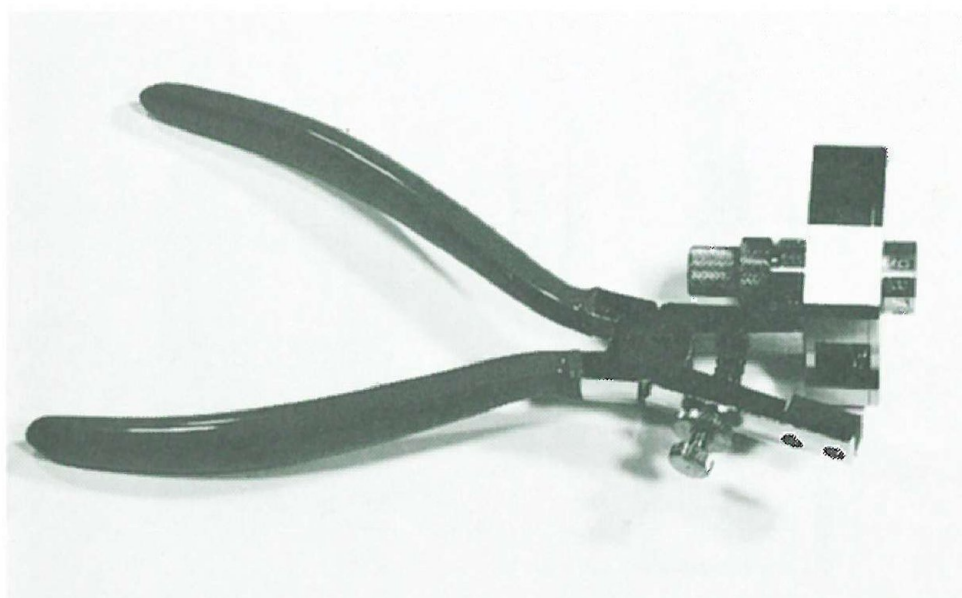


Figure 1e. The automatic pincers TZ 2L from E. Becker & Co. Ltd. with revolving needle magazine.

Figure 1a-e. Reproduced by the kind permission of Honma et. al. (1986).

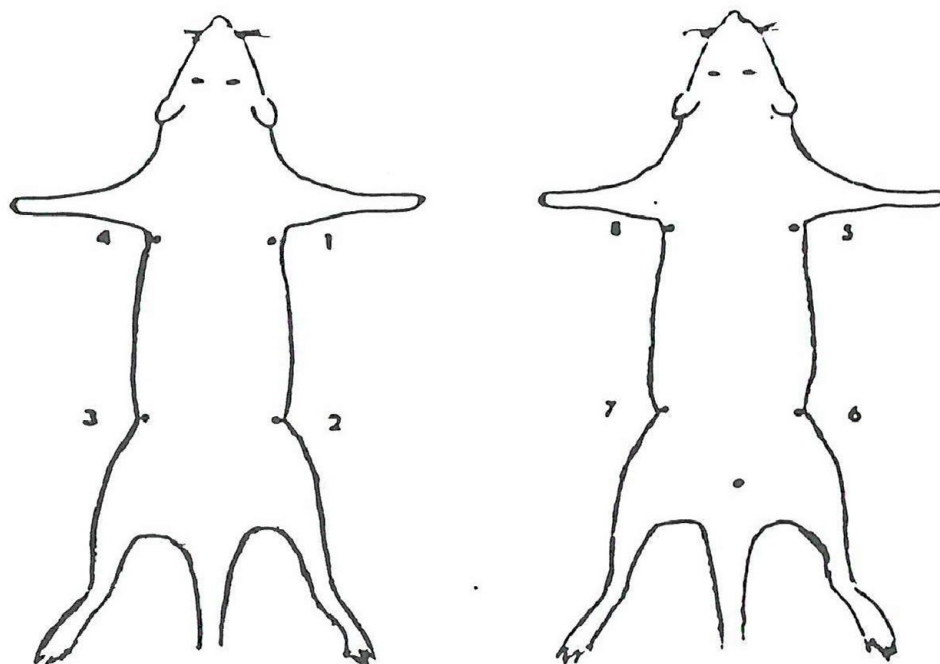


Figure 2. Tattoo numbering system for identifying new-born mice and rats. Number 1-4 require one mark, number 5-8 require two marks, one at the base of the tail. The fig. has been reproduced by the kind permission of Schoenborne (1977).

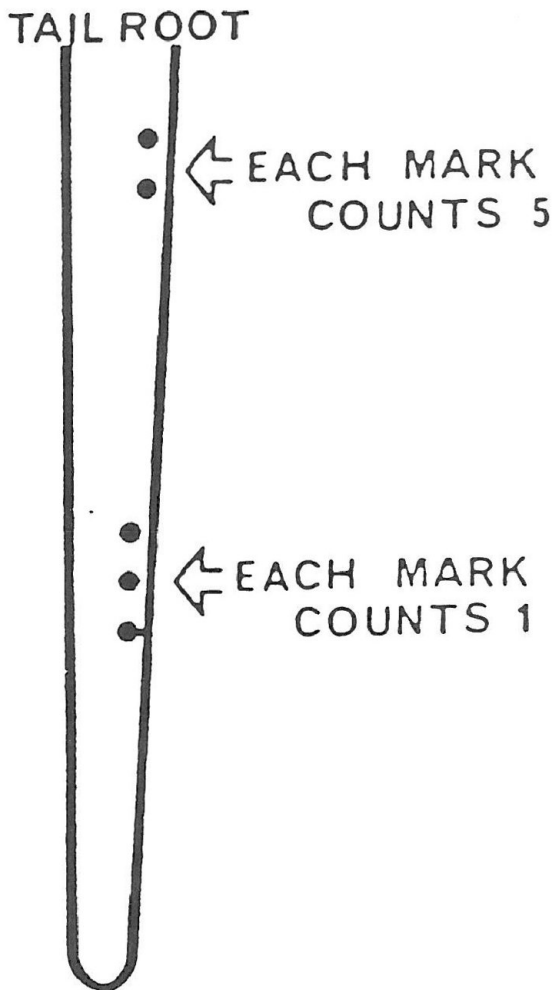


Figure 3. Tail-tattoo code for new-born mice. This tail is coded 13. This fig. has been reproduced by the kind permission of Greenham (1978).

Centre of Antibiotic Analysis in Washington D.C. in order to identify rabbits used for the pyrogen testing. Here, an electrically operated tattooing device was used; and, local anaesthesia was performed of the auditory nerve and auricular branch of the facial nerve. For this tattooing a specially designed cage for holding the rabbits was also used (Castor and Zaldivar 1973).

(3) Body-tattooing

Body-tattooing of new-born mice and rats can be performed within 24 hours after birth by spot marking with a tattooing machine and specific code (see Fig. 2). The tattoo should be placed in a fleshy area to ensure a better mar-

king. Also a small amount of ink is placed on the mother's whiskers so that she will accept her offspring (Schoenborne *et. al.* 1977).

This method does not increase mortality, nor does it seem to have a toxic effect on the animal. It is therefore recommended for use to identify individual newly born of the same litter.

(4) Tail-tattooing

The method is simple and has been used in new-born mice. It consists of injecting India ink paramedially through the skin into the underlying tissue of the dorsal tail surface using a sterile 25 gauge hypodermic needle. The midline is avoided so that the ink does not enter the coccygeal blood vessels. When tattooing a large number of mice, a tuberculin syringe is loaded with the ink. The needle is attached, and, the tattooing is accomplished by repeatedly recharging the needle for the syringe. A new needle for the syringe may be employed for each mouse.

An example of a marking code for this method is shown in Fig. 3, where each spot near the root of the tail gives a 5 point count and each spot near the midline gives a 1 point count.

This method leaves a permanent mark (life long) and, therefore, is a good method for identifying mice and rats. It is mainly recommended for a small number of animals (Greenham 1978).

(5) Ear-cuts or punches

Ear-cuts or punches are done using sterilized, sharp surgical instruments by experienced technicians who can complete the process quickly and easily (see Figs. 4, 5).

Ear-punching of small rodents with such instruments was described by Riley & Gwilliam (1981). The pain produced by this method is comparable to ear piercing in women for the attachment of ear-rings (Lane-Petter, 1967 and Wolff 1967).

A well defined code for ear-punching or cutting is required. It is advisable to display a code chart whenever marked animals are used.

The size of the ear-punch or cut should be distinguishable yet not too large in order to avoid



Figure 4. Ear punching technique & instruments from Riley & R. Gwilliam (1981).

Figure 4a. A method of ear punching.



Figure 4b. The hole left by the new punch.

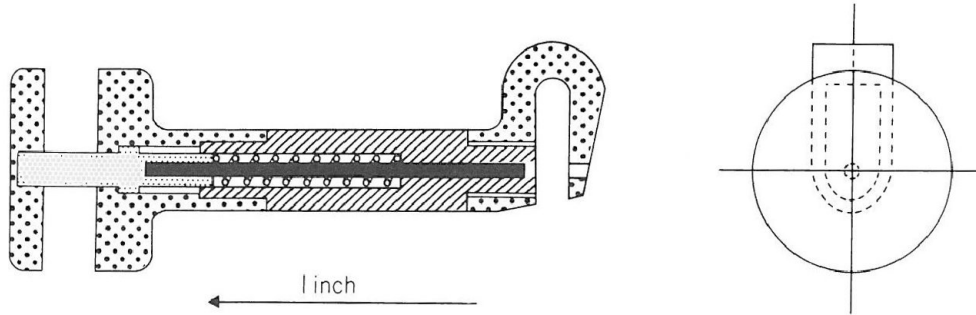


Figure 4c. The ear punch proposed by Riley & Gwillian which is made of stainless steel.

miss-shaping of the ear, and unnecessary discomfort when it is performed (see Fig. 6). Furthermore, to prevent infection and avoid irritation, the space between two cuts should have an adequate blood supply (Hafez 1970, Rugh 1968, Short & Woodnott 1969).

Ear-punching is a useful method for the identification of rats rather than mice as it may cause cannibalism amongst the mice. On the other hand, ear cutting is more suitable for mice. However, this method, in general, may be time consuming in monitoring the identification marks according to a code chart.

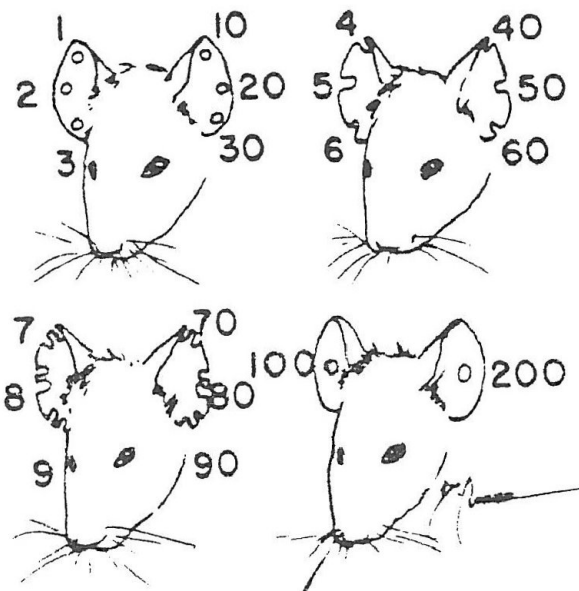


Figure 5. Ear notch punch code for identification of rodents. These number codes are used in various combinations to produce the desirable number. Reproduced with the kind permission of Harkness & Wagner (1983), and Lea & Febiger, Philadelphia, USA.

(6) Toe-clipping

Toe-clipping can be used to mark 4 day old mice or rats. The method requires cutting off the first joint of the toes according to a code chart (see Fig. (6)). It provides a quick, readily readable and permanent identification of the animal. However, it is inappropriate in regard to animal welfare and therefore not recommended.

(7) Ear-marking with studs

The ear stud may be of plastic, aluminum or brass material, containing a specific number; and, it is fixed to the animal's ear by a punch and stud forceps technique, which pierces a hole in the ear tissue by fixing and closing the stud in position. This method is useful and often used for rabbits and guinea pigs, but not for rodents. The disadvantage of this method is that the studs may be pulled out, and their loss is a frequent problem.

(8) Ear-tagging

Ear-tagging is quite similar to the ear-marking with a stud in that a plastic or metal label is attached to the ear of the animal except that this time clips are used to fix them. Ear-tags sometimes cause local lesions, bleeding, swelling and cutting to the ear. As these tags can be lost, this method is not a very effective one and is therefore not recommended.

(9) Ear-buttons

Ear-buttons, like ear tags, are easy to apply. They can be fixed to an ear with a special pair of pliers or with a hole punch and hand applied

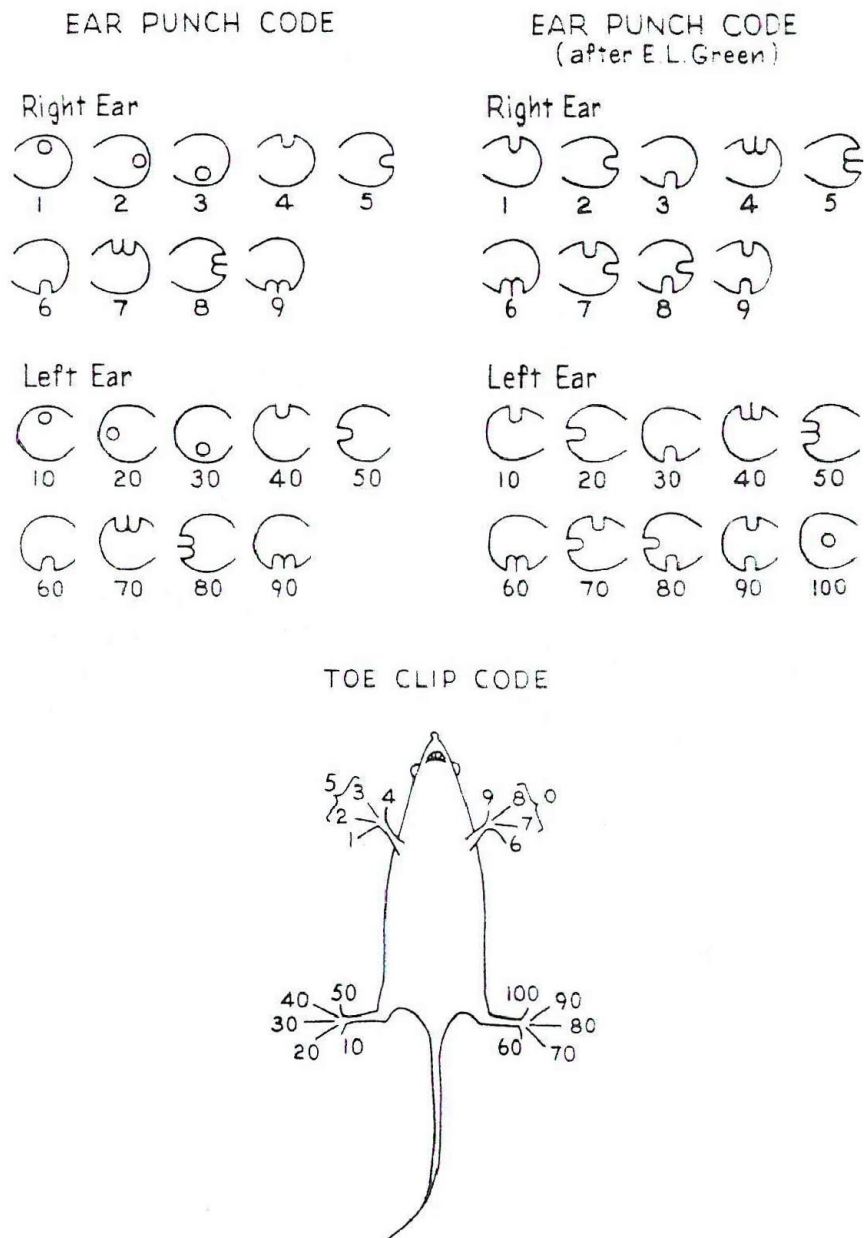


Figure 6. Identification code for mice from Wolff (1967). Reprinted with the kind permission from Churchill Livingstone Ltd., Edinburgh & London.

lable button. These buttons are available in various size and shapes, they are made of plastic or metal and offer little rejection and less problems. They have been successfully used with reasonable efficiency on guinea pigs and rabbits and to a lesser degree on some large rodents.

(10) Marking with rings

Rings are usually used for the identification of rabbits. Thus, a metal ring is placed above the hock joint of the hind leg. The ring contains the identification number of the animal.

(11) Freeze marking

Freeze marking is a new and popular method in the U.S.A. (Farrell & Johnston (1973) for mar-

king dogs and cats. However, it is felt that more research is required on it's application. The technique utilizes super-chilled refrigerants to produce white-hairs or balded marks on the animal's body, whereby the size depends on the time of application.

Methods of marking different animals

The method which is chosen for marking animals differs from one laboratory to another depending on a number of factors:

- (1) The type of test/research required to be performed on the animals.
- (2) The duration that the mark is needed for.
- (3) The age of the animals to be marked.
- (4) The experience of the person performing the marks.
- (5) The number of the animals involved.

The marking methods listed below are recommended for use in order of preference of each species, (naturally taking into consideration the animal welfare):

| Table 2 | |
|--------------|----------------------------------|
| Species | Marking methods |
| Rats: | 1 Back stain. |
| | 2 Tail-tattoo. |
| | 3 Ear-tattoo. |
| | 4 Ear- punch/cut. |
| | 5 Body-tattoo for young animals. |
| Mice: | 1 Back stain. |
| | 2 Ear-tattoo. |
| | 3 Tail-tattoo. |
| | 4 Ear- punch/cut. |
| | 5 Body-tattoo for young animals. |
| Guinea pigs: | 1 Back stain. |
| | 2 Ear-tattoo. |
| | 3 Ear- buttons. |
| | 4 Ear- punch. |
| | 5 Ear- stud. |
| Rabbits: | 1 Leg-ring. |
| | 2 Ear-tattoo. |
| | 3 Back stain. |
| | 4 Ear-stud. |
| | 5 Ear-tag. |

Summary

Identification of laboratory animals is an essential step for operating an animal house/research laboratory efficiently. Recently, strong trends towards the ethics of animal welfare of laboratory animals used in research has made it important to review some known methods of marking them. Thus, some reliable ethical methods of identifying animals used in the laboratory are recommended. Overall, this article addresses the above issue in mice, rats, rabbits, and guinea pigs and recommendation of various methods is given in Table 2.

Sammendrag

Artiklen er en oversigt over metoder til mærkning af forsøgsdyr. Gode og sikre metoder til mærkning af mus, rotter, kaniner og marsvin vurderes i forhold til deres anvendelsesområder.

Yhteenveto / K. Pelkonen

Koe-eläinten tunnistaminen on olennainen vaihe tehokkaassa koe-eläinyksikön/tutkimuslaboratorion toiminnassa. Viime aikoina on ilmennyt voimakasta pyrkimystä eläinsuojelullisempaan suuntaan koe-eläinten käsittelyssä, jonka vuoksi on ollut tärkeää tehdä tämä katsaus tunnetuista koe-eläinten merkintätavoista. Tämä artikkeli käsittelee hiiren, rotan, kanin ja marsun merkintää ja suosituksia näihin annetaan Taulukossa 2.

References

- Arrington, L. R.:* Introductory laboratory animal science. The Interstate Printers & Publishers Inc., Illinois, 1972, p. 153-158.
- Castor, G. B. and R. A. Zaldivar:* Tattooing rabbits ears for identification. *Lab. Anim. Sci.* 1973, 23(2), 279-281.
- Farrell, R. K. and S. D. Johnston:* Identification of laboratory animals: freeze marking. *Lab. Anim. Sci.* 1973, 23(1), 107-110.
- Farris, E. J. and J. Q. Griffith Jr.:* The rat in laboratory investigation. Hafner Publishing Co., New York & London, 1967, p. 13-17.
- Greenham, I. W.:* Tattooing new-born albino mice in life span experiments. *Lab. Anim. Sci.* 1978, 28(3), 346.
- Harkness, J. E. and J. E. Wagner:* the biology and medicine of rabbits and rodents. Eds. Lea & Febiger. Philadelphia, 1983, 2nd Ed., p. 3-5.
- Hafez, E. S. E.:* Reproduction & breeding techniques for laboratory animals. Eds. Lea & Febiger. Philadelphia, 1970, p. 282-283.
- Honma, M., S. Iwak, A. Kast and H. Kreuzer:* Experiences with the identification of small rodents. *Exp. Anim.* 1986, 35(3), 347-348.

Lane-Petter, W.: Animal-house equipment in The Universities Federation for Animal Welfare (UFAW), Handbook on Care and Management of Laboratory Animals. Eds. Churchill Livingstone Ltd., Edinburgh & London, 1987, 3rd Ed., p. 60-61.

New, A. E.: Identification of small animals-procedures and problems. Clin. Toxicol, 1978, 13(5), 601-610.

Riley, J. and R. Gwilliam: A new ear-punch for small rodents. J. Inst. of Anim. Technicians. 1981, 32(1), 53-56.

Rugh, R.: The mouse: it's reproduction and development. Burgess publishing Co., USA, 1968, p. 296.

Schoenborne, B. M., R. E. Schrader and N. L. Canolty: Tattooing new-born mice and rats for identification. Lab. Anim. Sci. 1977, 27(1), 110.

Short, D. J. and D. P. Woodnott: The I. A. T. Manual of Laboratory Animals and Techniques. Eds. Charles C. Thomas, Illinois, USA, 1969, 2nd Ed., p. 34-39.

Stonehouse, B.: Animal marking, recognition marking of animals in research. Macmillan Press Ltd. London, 1977, p. 34-39.

Wolff, G. L.: Practical mating systems and record keeping in a breeding colony, in UFAW Handbook on the Care and Management of Laboratory Animals. Eds. Churchill Livingstone Ltd. Edinburgh & London, 1967, p. 107-110.

Annonce

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Motivet på kalenderposteren forestiller: Næroptagelse af iskrystaller på et vindue med solnedgangen som baggrund.



Vinderne udtrækkes af
Jet Møllegaard Landsfeldt

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Blandt de fantasifulde, men ikke korrekte svar kan nævnes: »Bronchoskopi af en asbest-lunge«, »Alger på bunden af havet«, »En udsigt set indefra et øje, som kigger udad«, »Lys-et set for enden af tunnellen« og »Håb«.