Somark is a global leader in laboratory animal identification, working to revolutionize preclinical research laboratories with innovative technology and products, including Labstamp and Digitail.

ANIMAL IDENTIFICATION METHODS - EXPLORING BEYOND GENOTYPING

Throughout this paper, we'll discuss the complex topic of genotyping, and all of its intricacies relating to compatible identification methods. By covering the different types of identification methods suitable for genotyping, with their advantages and disadvantages, we've highlighted how researchers can maximize animal welfare regulatory compliance and improve integrity of preclinical research data.

There are several methods available for research facilities to identify laboratory mouse models. From the more popular, permanent and invasive methods like ear notching, and toe clipping to temporary options such as fur shave, coat dyes and marker pens, each method has its own set of advantages and disadvantages to consider for your research institute.

In more recent years, new technologies have been created to try and combat the issues raised in regards to the welfare of the laboratory animal with some of the more permanent methods. Ensuring data integrity is maintained is also paramount, both factors of which are achieved with the less-invasive Digitail, and the non-invasive Labstamp from Somark Innovations.

Often when liaising with Laboratory Technicians and Research Program Managers, methods such as Labstamp or Digitail are faced with the feedback "I need to perform genotyping, so it makes sense to combine it with ear notching or toe clipping in a single procedure".

Throughout this report, our aim is to provide you with the bigger picture. Enabling you to make a more informed decision for both your facility regarding data integrity, and also for the welfare of the animals.

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Importance of Genotyping in Preclinical Research

The focus of this report is to divulge the importance of managing the preclinical research process, genotyping, along with the chosen identification method deployed. We will particularly focus on the common methods opted for and the consequential implications on laboratory mice and the study results data.

Genotyping is the name of the process that examines a living organisms DNA to evaluate its genetic makeup, or genotype, and highlight any specific variations.

Genotyping plays a critical role for a number of reasons, including:

Disease Models:
Genotyping helps in creating accurate disease models, which are essential for understanding human diseases and developing treatments.

Drug Development:
By identifying genetic differences, researchers can study drug efficacy and toxicity, tailoring treatments to specific genetic profiles.

Breeding Programs:
It ensures the genetic integrity of breeding programs by confirming the genetic makeup of animals, reducing the chances of genetic drift.

Identification of Genetic Markers:
It aids in the identification of genetic markers associated with certain traits or diseases, facilitating advancements in genetic research.

Studying genotype data in a preclinical setting can provide valuable genetic response insights into virus or disease development within humans, with genotyped mice being irreplaceable (as of yet) for bridging the gap between drug discovery and development research and clinical applications.

There are many different sample types that can be used during the genotyping process, and where possible, researchers are encouraged to opt for the non-invasive methods such as using blood samples, hair follicles, colonic cells or cells obtained from oral mucosa.

If a larger sample is required, ear notching, tail biopsy, or toe clipping are the alternative adequate assay samples; however all do come with a strict set of guidelines that need to be followed.

Although we understand its importance, and the convenience to take the necessary DNA sample whilst simultaneously implementing identification of the mouse, this article will discuss the importance of considering alternative methods of identification in order to achieve superior data accuracy as well as increased animal welfare compliance.

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Overview of Ear Notching

The practice of ear notching has been in regular use for over 75 years, and involves cutting small, uniquely patterned notches in the ears of rodents. This method has been widely used due to its low-cost and simplicity.

When conducting the application, each ear notch pattern corresponds to a specific identification code, allowing researchers to distinguish between individual mice in a study.

This method of identification is best performed on strains between 14-17 days of age and was the most predominate method of its time since it does not require anesthesia or analgesia. Many concerns have since risen about the pain for the rodent during this procedure. According to a research article by AL Miller and MC Leach, there has been very little pain assessment data collected during the process of ear notching even though it "is an important issue to address as pain not only compromises welfare, but also potentially the validity of the data collected from these animals." With animal welfare increasingly prioritized in pre-clinical research, this raises the question: why is there so little data on pain assessment with ear notching?

In 2015, the pair published further findings linking to the pain assessment data collected. This time they addressed the effectiveness of utilizing the mouse grimace scale (MGS) to assess pain associated with routine ear notching. The grimace scale focuses on specific facial action units and their presence in response to environmental behavioral of procedural changes. In this instance, this also included pain.

Using the same framework on other laboratory species, including rats and pigs, the MGS scores outlined results of pain. The same findings were not apparent in the study conducted on C57BL/6 male mice. Conclusions were drawn that there are limitations with using the MGS to assess mouse pain.

Due to the absence of adequate data to truly assess the pain of ear notching or the consequential risks this method poses to research data and the welfare of mice this practice is now gaining criticism for the pain it likely causes the animals. Additionally, it also relies heavily on human accuracies and mistakes can lead to misidentification.

Overview of Toe Clipping

Toe clipping involves the removal of one or more toes from a rodent's foot, usually within the first 7 days of life and only if no other identification methods are available. This practice is now largely out of favor and comes with numerous restrictions. The University of Connecticut states in their approved policy guidelines that toe clipping in their facility cannot be used solely for DNA sample collection.

It is also not uncommon for policies to state that only a single procedure may be performed, if a DNA sample is needed for genotyping along with implementing an identification method.

Inadequacies of Traditional Methods

Arguably convenience has been favored over welfare and legitimacy in research data in years gone by, as genotyping procedures were bundled together with notching and clipping in order to efficiently process mice and begin research with a more immediate effect.

There's no dispute that two procedures at the forefront of study preparation will extend the time taken to begin research. With evolving guidelines likely to rule out the single use of toe clipping, it is also expected that regulatory bodies will one day hone in on other methods in the future. This only highlights the importance of adapting to the likelihood that identification processes should suit the mouse, not the researcher.

The argument lies in the long term, that the risks to the welfare of the laboratory mouse may actually harm credibility of the research program.

This is where methods like Labstamp and Digitail offer significant benefits by reducing the need for frequent handling throughout the animal's life. Additionally, the ease of identification helps to mitigate data inaccuracies associated with other methods.

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Animal Welfare Concern

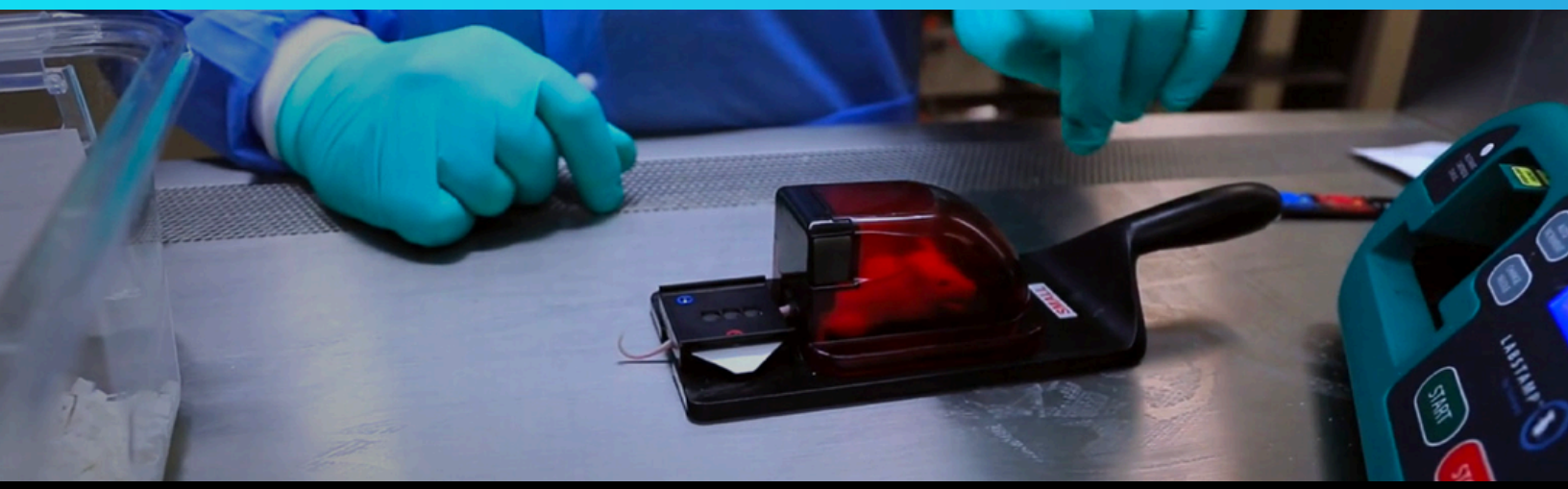
Concerns have been raised about the impact of many identification methods on animal welfare. For example, ear notching has been shown to cause significant distress. A study published in the British Veterinary Association Journals reported an instance where a mouse "caught its left foreleg through its left ear punch up to the level of the shoulder." It is incidents like this that highlight the welfare issues associated with such methods.

Institutes such as UCLA and UCONN, to list a few, have stated that toe clipping can only be used as a last resort, as per the recommendations from Guide for the Care and Use of Laboratory Animals which states, "as a method of identification of small rodents, toe-clipping should be used only when no other individual identification method is feasible." As such, the Interagency Research Animal Committee stated that top clipping is considered a painful procedure.

The welfare concern with ear notching is that a) it's painful and b) it requires excess handling every time you want to read the mice. Labstamp has extended visual read distances, through glass and plastic too. According to a study published by a global pharmaceutical company, it found that Labstamp offered dramatically increased identification efficiency, as can be seen by these below results:

- 78% quicker animal identification compared to ear notches
- 27% quicker identification compared to metal ear tags

Whilst it's cited in a number of journals, that some methods of tattooing rodents can cause a level of pain, the Labstamp has been designed to avoid tissue trauma, dermatitis and auricular chondritis. The tattoos and its associated application method have received independent ethics approval from the IACUC.



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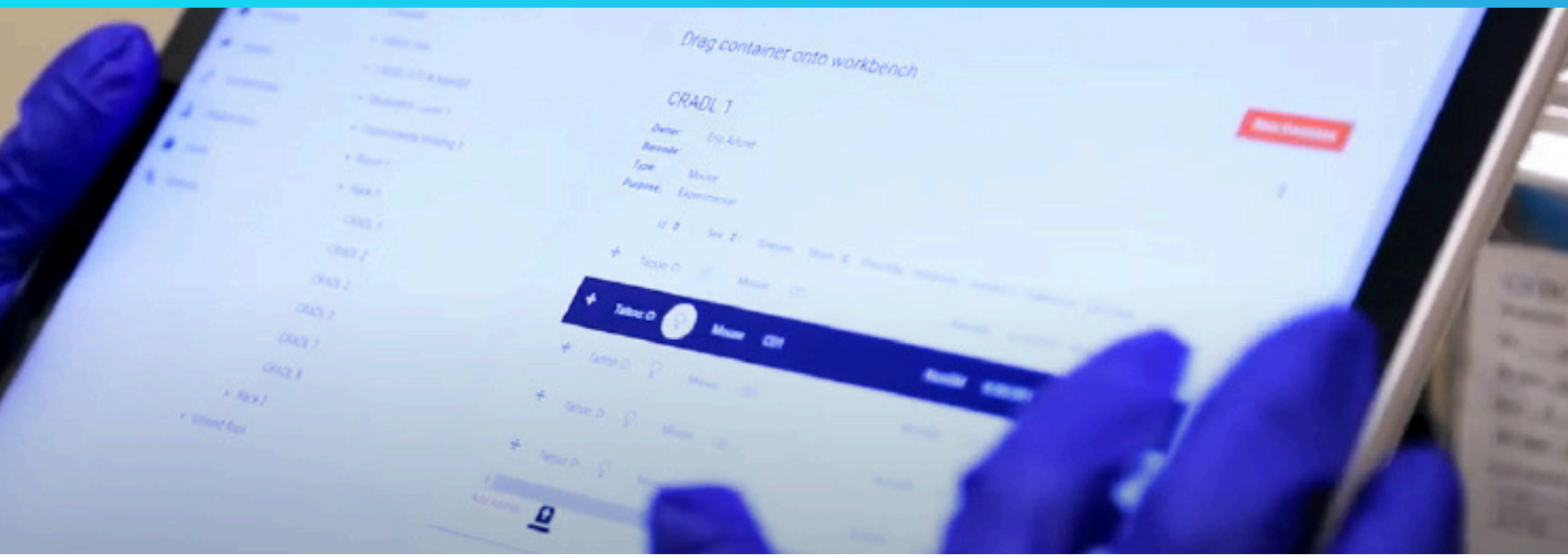

Data Accuracy Issues

In addition to animal welfare concerns, a major issue with toe clipping and ear notching identification methods is the potential for data inaccuracies. A study from Journal of the American Association for Laboratory Animal Science found that "at least 25% of tag codes were misread" when compared to other identification methods. These instances of misidentification or errors in reads emphasize notching and clipping as a cause of significant discrepancies in research data.

Unlike the Labstamp, which has been engineered to last the lifetime of the mouse by depositing microencapsulated ink into the mid-dermal layer of the mouse's tail, alternative identification methods such as ear notching, aren't as precise. Notches can be torn if snagged on apparatus. "In addition, fighting can also cause ear notches to become indistinct or torn, leading not only to additional harm but less confident identification" as stated by Johnny V Roughan and Tatum Sevenoaks in their recent study.

This harm, along with the stress caused by handling the laboratory mice, poses "arguably the greatest potential to damage studies [yielding] the possibility of misidentification."

Excessive handling and invasive methods are directly linked to increased stress in mice. Mice exhibit behaviors that are not accurate or true representations of their natural state when experiencing stress and anxiety. These stress-induced behavioral changes in preclinical research studies can result in unreliable data. This is particularly true of behavioral testing in toxicology for example, where the cause of behavioral changes can make it difficult to draw valid conclusions from the research data.

The use of less invasive applications of identification should minimize stress, through both a significantly reduced need for animal handling during the application and the ongoing reading of identifiers, helping to maintain the scientific integrity of preclinical studies.

Alternative Identification Methods and Genotyping

As previously mentioned, we are no longer and should no longer be restricted to choosing between two highly invasive methods like toe-clipping or permanent punched markers for mouse identification. Recent advancements in identification technology have introduced new, less invasive options.

We anticipate that genotyping too will experience an overhaul by ethical guidelines, with genotyping sample collection methods such as blood sampling, already rising in popularity with research institutes. In order to ensure that research progression and preclinical trials are approved by the FDA, researchers should begin to investigate new methods of identification, rather than leave it too late.

By using non-invasive methods, such as those listed below, in conjunction with genotyping, we can ensure minimal risk to animal welfare during a study, as well as the generation of the most accurate research data.

Non-Invasive Identification Techniques

Within the scientific sector, there are several less invasive identification methods, including fur shaving, coat dyes, marker pens, RFID tags like Digitail, and tail tattooing systems like Labstamp. This range encompasses both low-cost, temporary solutions and more advanced, permanent options, catering to various needs and budgets.

Whilst techniques such as coat dyes, or fur shaves have their place within the scientific field, the impracticality of their temporary status means they aren't frequently used within preclinical research. Instead, more accurate, permanent identification methods are required, to help substantiate any findings made.

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Labstamp

The Labstamp method requires minimal training and offers a fast and efficient process for identifying laboratory mice. The whole process of safely restraining and tattooing the laboratory mouse takes less than 30 seconds, so any discomfort that could be experienced by the mouse is minimal. The tattoo provides no side-effects or long-term stress inflicted on the mice for the duration that they need to be identified.

There are over 3+ million mice tattooed with Labstamp, from albino and pigmented to nude strains, with no customer having reported any health issues caused from this ID method.

One of the major benefits of using Labstamp is the limited animal handling required, made possible by Somark Innovation's commitment to improve animal welfare which lies at the core of all their identification methods.

Beyond placing the mouse into the safety restraint, there is no additional need for animal handling, thanks to the read distances being greatly extended with Labstamp, with little to no errors, as highlighted in a study published by a global pharmaceutical company, who found the following identification errors when comparing various identification methodologies:

- Ear tags – greater than 30%
- Ear notches - greater than 20%
- Labstamp tattoo - less than 3%

The same global pharmaceutical company also found that Labstamp provided 78% quicker animal identification compared to ear notches, and 27% quicker identification compared to metal ear tags.

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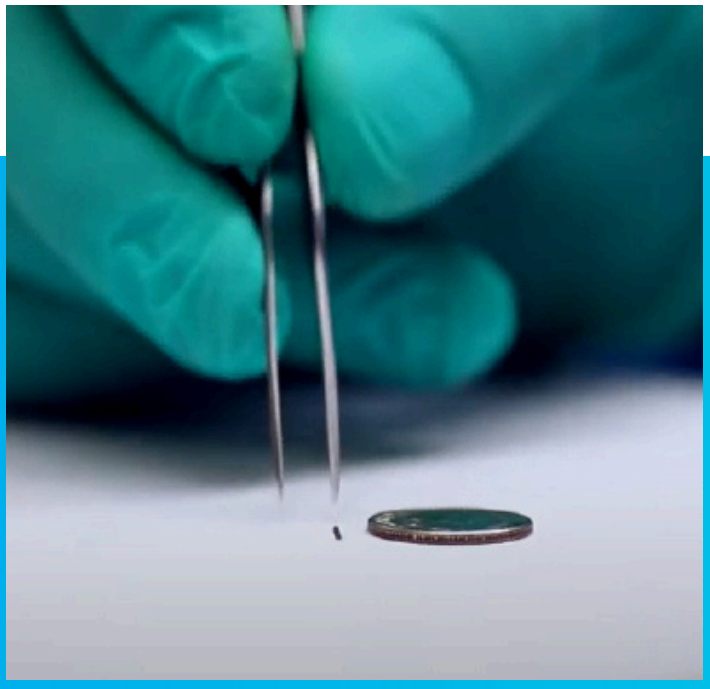
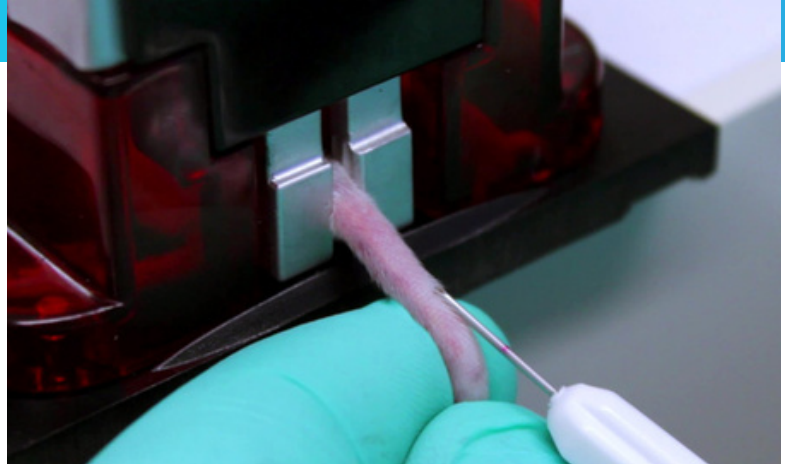
Digitail

Specifically designed for rodents, the R&D team have considered all aspects of the welfare of the animal and the data collected to purposefully design the world's smallest implantable RFID identification tag. This minimally invasive procedure uses a 21-gauge needle, significantly smaller than the typical 16-18 gauge needles, making it the least invasive tag in the world.

The Digitail RFID tag is easy to implant by even novice technicians, and can be injected subcutaneously directly into the base of the tail, with little to no pain experienced by the mouse. There are no migrations with the Digitail RFID tag, allowing for unmatched data accuracy and no misreads. The tag also offers reliable read distance of up to 5cm, with the ability to read the tag through glass or plastic enclosures.





As highlighted with the Labstamp, there is minimal animal handling required thanks to the specially designed restraint. The tag is quick to insert, virtually painless, and leaves very little impact on the animal.

The secure restraint allows the tail to be accessible to the technician, whilst leaving the mouse free to comfortably move within the red dome. Unlike traditional tube restrainers, the tail cannot be pulled away from the technician. The aim of the Digitail restrainer is to provide a better experience for both the mouse, and the technician. The mouse experiences reduced stress, and the technician has to minimally handle the mouse, allowing for a quick and easy job.

The Digitail system is highly scalable and can generate an infinite number of unique IDs, making it ideal for both small and large-scale studies. In a recent 5-week study involving 20 Balb/C mice, where daily observations were made to track ID and weight, the Digitail RFID tag demonstrated:

- 49% quicker - Improved average mouse selection and weight collection from 33 to 16 seconds
- 100% accurate data collected and recorded for the right animal every time

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Ethical and Regulatory Considerations

Ethical Implications

Choosing an animal identification method is a critical decision for organizations and researchers, as it directly impacts the health and well-being of their animals. Consequently, several ethical responsibilities need to be considered.

1. Minimizing Harm – choosing methods which limit long term effects and implement minimum suffering in order to generate required results.

2. Accuracy and Reliability – as with all empirical research, the methods must produce data that has integrity, and the identification method has to be reliable to offer data validity. This is a common reason why institutions are more frequently using methods such as Labstamp or Digitail. Other identification methods such as ear notching can be confused or become illegible meaning the durability of the data can be questioned.

3. Regulatory Documentation of the process chosen and all of the considerations undertaken are essential to satisfy organizations such as IACUC and AAALAC.

4. Reporting and Transparency – Institutions have a responsibility to openly declare the method of identification that they have chosen. They should publish reports justifying their selection and acknowledging any potential limitations.

Regulation Landscape:

Institutions such as IACUC (Institutional Animal Care and Use Committee) and AAALAC (Association for Assessment and Accreditation of Laboratory Animal Care) do not enforce specific regulations like a government agency would. However, they do require institutions to demonstrate the use of good practices in several areas, as outlined below.

Animal Care:
AAALAC assesses whether institutions provide adequate space and ventilation for animals and ensure that qualified, experienced staff are responsible for their well-being. Additionally, institutions must have processes in place to demonstrate a commitment to minimizing pain and distress for the animals.

Protocol Review:
IACUC specifically reviews research and teaching protocols to ensure the justification for animal use and the scientific merit of their inclusion. If IACUC identifies non-conformance to their protocols, they have the authority to submit reports and findings to other institutions and regulatory agencies, advocating for changes.

It is important to note that organizations such as AAALAC often implement guidelines that exceed government regulations, ensuring higher standards of animal care and use.

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SOMARK CELEBRATING 10 YEARS OF Pioneering Animal Identification

As highlighted throughout this article, there are several critical considerations when pairing an animal identification method with genotyping; convenience is no longer an acceptable argument. Primarily focusing on animal welfare and the integrity of the data collected, clipping and notching are traditional methods that now raise significant ethical and practical concerns. These methods can cause stress and pain to the animals which simultaneously compromises the validity of research data.

The advancement in identification technologies, such as the Digitail RFID tags and the Labstamp tattoo system, offers less invasive and more reliable alternatives. These methods not only enhance animal welfare by minimizing handling and discomfort but also improve data accuracy and reliability, which are crucial for preclinical research.

Both methods are developed by laboratory professionals specifically for preclinical research, with careful consideration of animal welfare and its potential impact on the trial, as well as the integrity of the recorded data.

Ethical responsibilities in choosing identification methods are paramount. Researchers and institutions must prioritize minimizing harm, ensuring accuracy, and maintaining regulatory compliance. Transparent reporting and justification of chosen methods are also essential to uphold the integrity of research.

By considering these factors, researchers can make informed decisions that align with ethical standards and scientific rigor, ultimately contributing to more humane and effective research practices in the scientific community.

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