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Use of grip strength meter to assess the limb strength of *mdx* mice

SOP (ID) Number	DMD_M.2.2.001
Version	2.0
Issued	September 3rd, 2008
Last reviewed	March 11th, 2014
Author	Annamaria De Luca Sezione di Farmacologia, Dipartimento Farmacobiologico, Facoltà di Farmacia, Università di Bari, Italy
Working group members	Jon Tinsley (Summit plc, UK) Annemieke Aartsma-Rus (Leiden University Medical Center, Department of Human Genetics, Leiden, The Netherlands) Maaike van Putten (Leiden University Medical Center, Department of Human Genetics, Leiden, The Netherlands) Kanneboyina Nagaraju (Children's National Medical Center, Washington DC, USA) Sabine de La Porte (CNRS, Gif-sur-Yvette, France) Judith Dubach-Powell (Santhera Pharmaceuticals Ltd, Switzerland) George Carlson (Dept. Physiology Kirksville College of Osteopathic Medicine Kirksville, MO. 63501-1497, USA)
SOP responsible	Annamaria De Luca
Official reviewer	Kanneboyina Nagaraju

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1. OBJECTIVE

The grip strength test is a simple non-invasive method designed to evaluate mouse muscle force *in vivo*, by taking advantage of the animal's tendency to grasp a horizontal metal bar or grid while suspended by its tail. The bar or grid is attached to a force transducer, and the force produced during the pull on the bar can be repeatedly measured at intervals (e.g., weekly) during a given experimental period. Because of its simplicity and economy, the grip bar strength dynamometer is the most commonly used *in vivo* test for monitoring impaired limb strength (fore and/or hind limb) caused by pathology progression and/or chronic exercise in *mdx* mice and for determining whether therapeutic interventions (drug, gene and/or cellular) can reduce muscle weakness in dystrophy.

2. SCOPE AND APPLICABILITY

The purpose of this assay is to assess the animals fore and hind limb strength. The method can be used to measure the disease progression as well as to test effect of specific therapeutic interventions in mouse models of neuromuscular disorders. The grip strength test enables the performance of the muscular apparatus in conscious dystrophic mice and the effect of various experimental interventions to be assessed. Mice that are older than 2-3 weeks of age are generally suitable for this test.

Advantages

Because this test is non-invasive and does not damage the muscle, it can be performed longitudinally throughout most of the lifespan of dystrophic mice. Therefore it is a useful tool to assess the effect of either a short or long-term treatment. When the test is performed in a fashion that maintains rigorous timing, the animals do not lose interest in the test over time.

Disadvantages

Duration: Since the test has to be performed on one mouse at a time, with at least 1 min elapsing between each of the five determinations per animal (see below details about the procedure) the testing requires several minutes per animal. This leads to a considerable time investment, especially when several experimental groups are being concurrently assessed.

Variability: Because this is an *in vivo* test, it shows a certain level of variability and requires time and attention to achieve the best results. In standardized conditions, variability within a group of animal of similar gender, age, and genotype should range around 10-25%, a similar variability being observed among the five determination performed in the same animal during the trial. Greater variability may reveal a bias in the test (animal fatigue, not proper procedure, external source of variation such as light, temperature, noise, etc). The variation between two independent (and expert) experimenters can be as large as 30%, which however constantly apply throughout the test. For this reason it is critical that the same experimenter monitor mouse strength for all experimental groups under study and for the entire duration of the experimental session - either short or long term sessions.

Learning/habituation bias: As for any behavioural test, it is recommended that the number of iterations in each session be limited and that the experimenters allow for a sufficient lag time between successive sessions to avoid learning bias (e.g., at least 5-7days). One can assume that when the procedure is repeated at a certain frequency, the animals generally learn the protocol and lose interest in applying resistance to maintain their gripThen, it can be assumed that determination of grip strength on weekly basis would ensure unbiased results.

Discrimination between muscle performance/learning ability/general behaviour: This test primarily measures limb force, but it may also be affected by the relative amount of lean/fat mass, the learning ability of the mice, the state of circulatory and neuromuscular system as well as other environmental factors. Thus, a positive value observed for a drug treatment in this test should be confirmed by another more specific test (e.g., Force contractions on isolated muscle), since improvement in muscle strength by itself is not sufficient to conclude that a drug induces a muscle-dependent effect. Similarly, a negative outcome in this assay does not necessarily mean that the drug lacks efficacy on muscle (but this is more difficult to resolve).
Body weight should be measured in parallel, so that values can be normalized for strength/body weight for each mouse at the various time points.

3. CAUTIONS

The grip strength determination has to be performed under strict experimental conditions, since it may be strongly affected by multiple variables (e.g., volition, cognition, and fatigue) that are independent of muscle dysfunction. Therefore, as for other behavioural approaches, the benefit of an intervention may not be detectable as a direct effect on the muscle *per se*.

It is important that

- The same operator measures all the mice in a group in order to minimize human variability

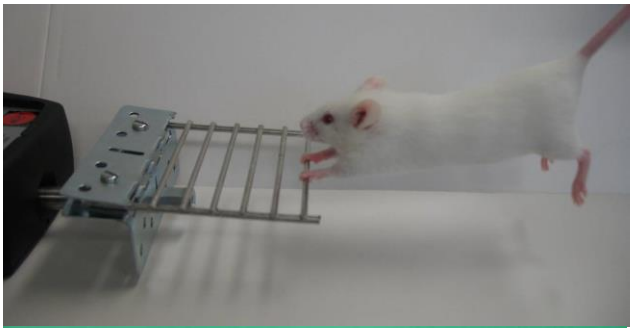
- Tests are preferably performed in a blinded fashion, i.e. the experimenter should not be aware of experimental groups (treated or not, wt or *mdx*) the mouse belongs to avoid personal bias.
- The test should always be performed at the same time of day, on the same day of the week (see above in relation to learning/habituation bias), and in a protected environment in order to avoid the potential influence of light, noise, and other variables.
- A group of normal mice need to be evaluated to assess the influence of the experimental protocol or of the environment on the change in force that is observed during testing (Variations in the animal housing, the season of the year, and the food provided can cause variability of the results). This does however have a negative impact on the economics of this test.
- Large groups of mice (6-8) that are of similar age and sex need to be used. If possible, control and test *mdx* animals should be littermates.

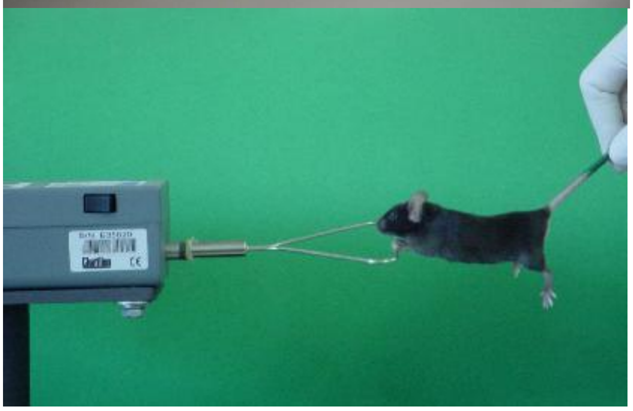
4. MATERIALS

The basic material needed is a digital force meter equipped with precision force gauges to retain the peak force applied on a digital display and with a grid or wire system that allows mouse grip by either or both paws. Data can be collected either manually (reading the values on the display) or on-line through a RS232 connection with a computer. A number of different force meters for the determination of grip and limb strength are commercially available (PanLab, Ugo Basile, Columbus Instruments, TSE Systems, etc). However they are not considered to be technically equivalent as there are major differences in design and electronics. A grip strength meter with an axial force transducer is to be preferred to one with a lever-type force transducer (the results of the later is easily inferred by the physical law of the lever).

- All commercially available force meters are digital dynamometers produced by different brands and re-sold for the specific animal use by biological research companies. These may re-sell it either directly or via local country specific representatives, with prices in the order of 1200-3000€ (for meter and grid/wire, based on direct or mediated sell, money exchange rates, taxes etc). Few commonly used models are listed below:
- Chatillon® DFE Series available with capacities from 2 lbf (1 kgf, 10 N) accuracy of better than 0.25% full scale; AMETEK TCI Division • Chatillon Force Measurement Systems; Largo, Florida (USA); re-sold by Columbus Instrument, USA
- CENTOR Easy force meter, 25N maximal force (see figure below); CatNo: CNR EA 25; produced by Andilog Technologies SA, Chaville, France; re-sold by PanLab and others

Grid or wire for the grip strength measurements (specifically designed grids (grids only!) are sold by PanLab for 350€, however can easily be assembled from materials which can be purchased from a local do-it-yourself store)

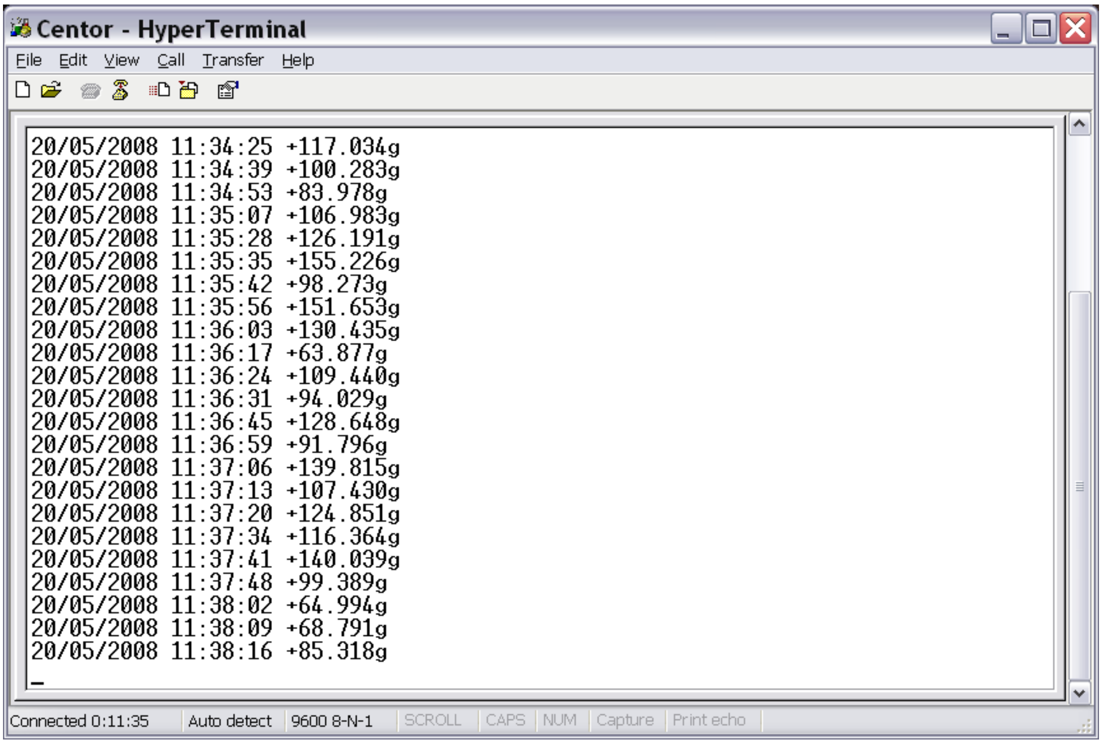




1) Custom made computer cable for RS232 connection (optional, for the direct transfer of data to a computer). The cable terminal diagram is the following:

Centor	Cable	Computer
SubD 15 pin, m	Function	RS232 (9 pin SubD, f)
3	RS232 RxD	2
4	RS232 TxD	3
13	Shield	5
shield	Shield	shield

2) A software (optional for the direct transfer of data to the computer; only in conjunction with the cable) such as HyperTerminal software for the PanLab meter (data transfer settings; 9600 Baud, 8 data bits, 1 stop bit, no parity, no flow control; as part of the Windows operating system the HyperTerminal program is free of charge). Generally, after acquisition and for further processing, the results can be saved as a text file or can be copy-pasted into any other application, i.e. Excel thus allowing easy management and statistical analysis of the original data. Using the optional cable and software, the data are transferred directly to the computer, along with a date and time stamp. This ensures the best possible traceability and avoids possible writing or typing errors.



Screenshot of the data as can be seen, if the data are transferred from the Centor Easy force meter to the HyperTerminal program. Similar data sheet can be obtained with other software for data acquisition.

Special comments:

The proper functioning of the force meter can be verified by exerting a defined maximal force on it as follows:

a) affix a paper clip to the grip bar (aim: paper clips are magnetic, the grip bar is usually made of stainless steel and is thus not magnetic)

b) attach a magnet to the paper clip in a defined manner (ideally the contact to the magnet should be just on one edge of the paper clip (not on the side) and the place of contact on the magnet should be also defined: this is easier if you use a small magnet).

c) with a slow, constantly increasing force, pull the magnet until the paper clip is released. If the contact between the paper clip and the magnet is sufficiently defined, the force necessary to break the contact will be relatively constant.

First, perform the check with the magnet in the middle area of the bar: constant values with a data spread of <2% should result. A larger spread of the data will indicate that the electronics are not functioning appropriately (the electronics of some grip strength meters are inappropriate, i.e. Ugo Basile). If the first test was successful, then repeat the same test first on the left and then again on the right side of the bar: the results should be the same, independently of which side of the bar the measurement was made. A difference in the measurement values from the left and right side indicate that there might be a lever-effect: some of the marketed grip strength meters are error prone due to the selection of a force transducer where the results are dependent on the animal's holding position on the bar. Therefore, such force transducers (e.g. the one from Ugo Basile) are not reliable. It is sufficient to perform these tests just once, on purchase and installation of the device.

The diameter of the pulling bar is also of particular importance. A very thin pull bar (as that of Ugo Basile) may allow a very tight grasp that requires a very strong pull to break it. In addition, the use of a very thin bar or wire could result in an animal wounding its paws. A very thick bar could result in weak grip strength values. A suitable bar should be 1-2 mm in diameter and composed of non-flexible metal, allowing an efficient grasp that can easily be broken by the operator.

The force meter has to be fixed to a firm base (so to be stable during the pull and not move as a result of mouse grasp), at a defined height above the table or surface. Positioning the force meter on the edge of a desk or table-top is a good idea, as upon breaking the grasp, the mice are then not likely to hit the top of the table.

The force meter has to be configured for the recording of the maximal force according to the manual and in the units (lb, kg, N) preferred by the experimenter.

5. METHODS

Force transducer meters are have to be set to zero or reset before each measurement is made so to allow proper values to be detected.
At least 6 to 8 mice per group are generally needed if statistical significance is to be reached for this parameter.

Fore limb grip strength measurement:

The most frequently used configuration is to measure *fore limb* grip strength.

1. Reset the meter. Choose g (grams) as unit/scale of values.

2. Lift the mouse by the tail to the height where the front paws are at the same height as the bar
3. Move the mouse horizontally towards the bar until it becomes within reach
4. Visually check that the grip is good i.e. a symmetric, tight grip with both paws and exerting a detectable resistance against the investigator's pull
5. Gently pull the mouse away until its grasp is broken. The pulling should be at a constant speed and sufficiently slow to permit the mouse to build up a resistance against it. The transducer saves the value at this point. Measurements must be discarded if the animal uses only one paw or also uses its hind paws, turns backwards during the pull, or leaves the bar without resistance.
6. Repeat the test a set number of times (but no more than 5 times) to obtain the best performance
7. Determine the body weight

The single best recorded value, or the three best, or a mean of all values can be used for analysis. For this purpose, attention should be paid to the spacing of the determinations, with enough time (at least one minute) between trials to allow the mouse to recover and to avoid habit formation.

Determination of fatigue:

As an alternative approach, repetitive tests can be performed in sequence with a short latency between each test (i.e. two series of five pulls each with a pause of 1 minute in between the series), and the reduction in strength between the first and the last determination can be taken as an index of fatigue.

Hind limb grip strength measurement:

For hind limb strength, generally, an angled mesh assembly is used for this purpose. Mice are allowed to rest on the angled mesh assembly, facing away from the meter and with its hind limbs at least one-half of the way down the length of the mesh. The mouse's tail was pulled directly toward the meter and parallel to the mesh assembly. During this procedure, the mouse generally resists by grasping the mesh with all four limbs. The pulling continued toward the meter until the hind limbs released from the mesh assembly.

Whole limb force measurement:

Another configuration is related to the use of a special grid that allows the mouse to grasp it with all four limbs. In this case, all-animal strength is measured. As the grip is generally harder to break, the pull applied is generally greater, with the possibility of increasing the stress experienced by the animal during the test.

Data evaluation:

At least 6 to 8 mice per group are generally needed if statistical significance is to be reached for this parameter.

The single best recorded value (Max Force), or the three best, or the mean value of each session can be calculated for analysis. In addition, the results can be expressed as a multiple of the body weight by dividing the grip strength value by the body weight of each mouse. For longitudinal studies, time-dependent variation (increment/decrement) with respect to initial values can also be used to evaluate effect of pathology/therapeutic strategies.

6. EVALUATION AND INTERPRETATION OF RESULTS

Determination of mouse strength *in vivo* requires careful attention and rigorous methodology in order to avoid introducing artefacts. During the first determination, the mice sometimes display an initial period of little participation in the test that reflects their learning process. Thus, the full test may need to be repeated 1 or 2 days later. Thereafter, the test should be repeated no more than once a week, to avoid habituation bias.

Usually wild type and *mdx* mice have fore limb strength values that are about five to seven times their body weight. Normally, *mdx* and wild-type mice have similar values of absolute and/or normalized strength that show an age-dependent increase between 4 and 12 weeks of age; some controversy does exist about non-exercised *mdx* being weaker that wt, with differences between laboratories possibly related to the different experimental conditions and/or recording apparatus. As mentioned, both the absolute and normalized values and their increment with respect to the starting point can be used for analysis. Values for absolute strength generally range from 0.09 to 0.120 kg at 4-5 weeks of age and may reach 0.150 to 0.200 kg at 12-15 weeks. The increment in normalized strength values is usually 0.95 to 1.5 after 4 weeks. A decrease in strength can be observed in *mdx* mice as a consequence of chronic exercise, with values generally being 30-40% lower than those of their un-exercised counterparts. In this case, the 4-week increment in normalized strength is markedly lower (< 0.5) or even negative. Certain variability may be present, depending on the experimental group and environmental conditions, with lowered strength values sometimes observed without exercise. Recent study reports fore-limb and hind limb grip strength values for mdx and control BL10 mice that underwent 30-minute run on a horizontal treadmill at 12 m/min, twice a week at 3 different age groups (10-12W; 20-22W and 38-40W) (Spurney et al., 2009). Drugs that positively affect muscle strength in exercised *mdx* mice can fully counteract the reduced strength increment or can even lead to an anabolic effect that can be detected, with a large increase in the normalized strength over the 4 weeks (De Luca *et al*., 2003; 2005; 2008; Burdi *et al*., 2009; Cozzoli et al., 2013).

Analysis

- Calculation of Max strength and Mean strength of individual mice
- Calculation of Normalised strength (Force/body weight for each mouse)
- Calculation of absolute strength increase over a time period (Force TX –Force T0)
- Calculation of normalised strength increase over a time period (NF TX – NF T0)

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