Background
Collection of semen samples from nonhuman primates is needed for a variety of purposes. Examples include reproductive research, species conservation efforts or assessing reproductive safety during drug development. The purpose of this guideline is to provide a review of appropriate approaches for the collection of semen samples and their applicability in select nonhuman primate species exclusive of evaluation and analysis of semen samples after collection. This guideline is not meant to be all inclusive but rather to present an overview of commonly used techniques in non human primate practices (e.g. preliminary assessment of maturity status in males or for evaluation of male reproductive endpoints during in-life study). Other semen collection techniques are available and may be considered when feasible and appropriate.

Semen collection approaches and applicability
A number of approaches for semen collection are available. Semen collection by vaginal washing after copulation (14) and by manual manipulation (masturbation) (7) have been described but are not commonly used in biomedical research. The most common approaches are electroejaculation and penile vibratory stimulation. Electroejaculation is the process of using an electric current to produce an ejaculate. The most frequently used electroejaculation procedure approaches are rectal probe electrostimulation and penile electrostimulation.

Rectal probe electrostimulation is generally reported to produce samples with lower sperm concentration than penile electrostimulation (2, 5, 17); it is therefore generally regarded as less well suited to quantitative evaluations of semen samples but is highly effective for qualitative analysis such as detecting presence of sperm in the ejaculate to confirm sexual maturity (11) or to assess the morphology of sperm cells (1). Penile electrostimulation is most frequently used in collection of sperm for quantitative analysis (e.g., determination of sperm count and/or concentration) (10, 12, 15, 16, 17, 19).

In smaller species such as Squirrel monkeys and Marmosets, penile vibratory stimulation has been shown to be the preferred method over electrostimulation (3,18,22)

a. Rectal Probe Ejaculation (RPE)
   a. Description

The RPE procedure is the historical method for semen collection in nonhuman primates (3,4, 6). The mechanism of ejaculation is by direct stimulation of peripheral neuromuscular junctions,
resulting in the contraction and relaxation of pelvic organs. This stimulation results in emission of the content of those organs.

The procedure requires sedation but does not require training. Once sedated, the animal is placed in lateral recumbency. The size of the probe is species dependent. It should fit comfortably into the rectum and the probe electrode footprint should be oriented directly over the prostate. Prior to use, the electrodes are lightly sanded, rinsed and sanitized. The penis is gently exteriorized from the prepuce and cleaned to remove particulate matter if necessary (e.g. sterile saline). The penis is held with powder free gloves during the collection procedure. The prostate is gently palpated to determine depth and to facilitate placement of the probe electrodes directly over the prostate. A thin layer of sterile water soluble lubricating gel is applied to the probe prior to insertion. To ensure contact of the electrodes with the prostate the tip of the probe is gently pressed downward by lifting up the other end of the probe. The probe is activated by slowly increasing the voltage until the animal responds to the stimulus by leg extension with or without clasping. The voltage setting that the animal responds to becomes the animal’s voltage set point (the lowest voltage that will elicit a response). Each stimulus is comprised of a slow increasing voltage to a response, holding it briefly and then decreasing the voltage. As the stimulus is applied the penis is observed for extension, rigidity, engorgement, and pre-ejaculate. Multiple repetitions comprise a set of stimuli. On average, three sets of stimulations will produce an ejaculation. During the entire course of stimulation the tip of the penis is held over a pre-warmed glass beaker. After ejaculation the sample is submitted for analysis.

b. Animal Welfare Considerations

This procedure is slightly more invasive than other techniques, and it is important to check the animal and the probe regularly to ensure that no blood is present. The rectum is gently manually palpated and checked for evidence of blood and to determine if there is an increase in internal temperature in the tissue overlying the prostate. If blood is present or there is an increased temperature above the prostate, the procedure is immediately stopped and the animal allowed to recover. Post-procedure care is up to veterinary judgment. The procedure should be limited to no more than twice weekly collection.

c. Animal Selection

Confirmation of spermatogenesis (by detection of sperm in semen) is the most reliable way to confirm sexual maturity in nonhuman primates (11). Prior to such confirmation, selection of males can be optimized by applying conventional selection criteria, such as age, body weight, and/or testicular volume (8, 9, 13, 20). Testosterone is not a reliable indicator due to its pulsatile/circadian fluctuations and large inter-animal variability (13). None of these parameters are unequivocally predictive of sexual maturity, and specific criteria for maturity are dependent upon the species and even the geographical source of the animals. For example, cynomolgus monkeys from Mauritius have been demonstrated to be sexually mature (sperm in semen) from
between 3-4 years of age, whereas those from mainland Asia required an additional year (>4 years old) to reach maturity (11). In general, nonhuman primates (cynomolgus or rhesus) less than approximately 4 years old and 4 kg body weight are not likely to be sexually mature (13, 20).

A physical exam of the animal prior to the procedure includes close examination of the rectum for abnormalities such as scarring, stricture or hemorrhoids.

b. Penile Electrostimulation Procedure (PEP)

a. Description

Penile stimulation consists of stimulation of afferent nerve endings in the penis, with subsequent anterior transmission of stimuli and stimulation of the efferent pathways associated with penile erection and ejaculation.

For this procedure, an alert (non-anesthetized) animal is placed in a primate restraint chair. Prior to the procedure, the male is acclimated on several occasions to chair restraint and may be acclimated to the procedures used for semen collection (e.g., application of gel/pads to the penis without stimulating current). The penis is gently extricated, gel defibrillator pads with lubricating gel are wrapped around the penis, and electrodes are attached to the gel pads and then connected to the electroejaculation stimulator device. Stimulating current is administered, the first pulsation given at low voltage (appropriate for the species and size of the animal) for no more than approximately 10 to 15 seconds, with slow incremental increases up to a predetermined maximum, and generally no more than 3 to 4 trials attempted at one session (depending on the specific procedure and the animal response). At each stimulation, the animal is carefully observed for physiologic response and ejaculation. The animal is stimulated until an ejaculate is obtained. A collection tube is placed at the tip of the penis to collect the ejaculated semen sample, which is then processed for evaluation of various parameters (e.g., ejaculate volume; sperm count, motility and/or morphology).

Semen collection can be performed multiple times in a row over days/weeks without ill effects; sperm numbers are maintained through several electroejaculations (21), but will eventually decrease with multiple collections. In consideration of both animal welfare and sperm production rates, the procedure is usually not attempted more frequently than weekly (e.g., during a 2 week prestudy period, for baseline determinations) and monthly/quarterly during the dosing phase of a study. The frequency of collection may increase depending on the study requirements.

Once animals are trained and experienced with the procedure, they should be observed carefully for possible spontaneous ejaculation prior to electrostimulation being applied.
Most reports of direct penile electrostimulations indicate higher numbers of sperm per ejaculate compared to the rectal probe method (5, 15, 17), and higher sperm quality based on the ability of sperm to fertilize oocytes in vitro (10). The direct penile method has been proven to achieve better stimulation of the entire reproductive tract and have fewer problems with urine contamination and semen sample dilution, due to the lack of anesthesia. Also, the potential for retrograde ejaculation into the bladder, which has been associated with the rectal probe method, is minimized with penile electrostimulation (17). This noninvasive technique does require the animal to be trained and the experimental environment to be controlled (e.g., quiet room, staff familiar to the animals). It may also take longer to obtain a sample with this method than with the rectal probe electroejaculation technique.

b. Animal Welfare Considerations:

Penile electrostimulation is a noninvasive procedure. Use of defibrillator pads and gel for application of stimulating current to the penis increases the animal’s comfort during the procedure and eliminates issues associated with older methods such as metal foil electrodes, which can lead to dermal trauma (16). Animals are not anesthetized for the procedure, but rather acclimated and trained for chair restraint. Stimulating current is administered in a conservative and carefully controlled manner. Even for animals that have experienced the procedure, no more than several attempts to collect an ejaculate would be attempted at one session on a given day. Positive reinforcement is given by rewarding the animal regardless of the outcome of the procedure. Animals with behavioral characteristics such as nervousness or anxiety that could cause duress to the animal and/or interfere with the success of the procedure are not selected for use.

c. Animal Selection

The animal selection process is the same as for rectal probe electro-stimulation.

c. Penile Vibratory Stimulation (PVS)

a. Description

Penile vibrostimulation is a procedure involving the natural reflex sequence resulting in a normal emission and ejaculation response, yielding a natural ejaculate (18). This technique uses a modified human vibratory device equipped with a collection vessel. The glass collecting tube has an internal diameter appropriate to achieve good contact with the penis and the required level of stimulation. It is used with the animal awake, and with positive reinforcement in a darkened room to reduce excitement of the animals. For collection of the sperm, the penis is gently extruded from the preputial sheath and the vibrating glass tube is turned over the tip of the penis and held against the preputial orifice (18). Vibratory parameters used depend on the response of the animal to stimulation and is increased gradually with allocation for rest periods. In
Marmosets, erection itself is not a good indicator of stimulation outcome. As an indicator of adequate stimulation, animals push their pelvis forward prior to ejaculation (18).

b. Animal Welfare Considerations

The use of electrical stimulation for semen collection in species such as squirrel monkeys and marmosets is possible (3, 14) but can be challenging. Animal size makes direct placement of electrodes for penile stimulation difficult. RPE is also less suitable (in terms of reliability and safety) because the reproductive and digestive anatomy of these smaller NHP species is accordingly of reduced proportions. Accurate placement of the rectal probe (especially during contractions) and delivery of a reproducible stimulus are difficult to achieve (18). Studies in squirrel monkeys and marmosets found significant increases in spermatozoa and accessory gland production in semen samples collected by vibratory stimulation compared to samples collected by electro-ejaculation (18, 22). PVS yields ejaculates of enhanced quality and combines the advantage of not requiring sedation, causing little to no discomfort and at the same time producing a natural ejaculate, free from contamination. From a practical point of view, the enhanced yield of motile sperm achieved with PVS in the marmoset is of particular significance in a species in which sperm numbers are naturally limited by the extremely small size of the ejaculate (18)

c. Animal selection

PVS seem to be mainly used in New World Primates, for example squirrel monkeys and marmosets (18, 22)
References