

## Chromatography Applications Laboratory Sparks Black Rhino Baby Boom at Chester Zoo

*“The Thermo Fisher Scientific customer applications laboratory was essential in helping us understand what we were looking at. We’re analyzing hormone metabolites in fecal material, not the traditional hormone, so we need to know exactly what we are measuring. We didn’t have the funds to purchase a chromatography system for our endocrinology laboratory. The Thermo Scientific applications laboratory provided the expertise and services we needed. They gave us a lot of scientific credibility, which enabled us to publish scientific papers. We are hugely grateful, it was beneficial to us.”*

*Sue Walker, Ph.D.  
Endocrinologist and Scientific Manager  
Chester Zoo, U.K.*

With an estimated 623 eastern black rhinos in the wild, they are not only endangered, they are critically endangered. The population fell 90% in just three generations from a dramatic surge in illegal poaching of rhinos for their horn which is used in traditional Asian medicine. The astonishing street value for rhino horns has accelerated their demise at alarming rates.

Currently there are approximately 240 eastern black rhinos living in zoos around the world. Every zoo that houses an eastern black rhino faces significant challenges when it comes to introducing a successful breeding

program. Chester Zoo in the UK, for example, had no births in a decade. If the species is going to survive, ideally each and every adult rhino should successfully breed to ensure a genetically healthy and sustainable future population.

Dr. Sue Walker, joined Chester Zoo as Endocrinologist and Scientific Manager in 2007. One of her main objectives was to understand why rhino breeding is so challenging and to introduce measures to breed rhinos more successfully. In 2007, Chester Zoo established its Wildlife Endocrinology Laboratory with a full-time wildlife endocrinologist. Chester was



the first UK zoo to have its own dedicated endocrinologist and wildlife endocrinology laboratory.

Dr. Walker, who heads up the laboratory had previously completed her master’s thesis at the University of Guelph (Canada) on the red wolf species survival plan (SSP) in collaboration with Toronto Zoo.

She had also worked in the Reproductive Physiology unit at Toronto Zoo (Canada) and the Smithsonian Institution (USA) before moving to the U.K. to get a Ph.D. at the University of Liverpool, studying the effect of stress on reproductive health.

## Assessing Hormones for Reproductive Analysis

When animals fail to thrive or breed, clues can generally be found by assessing the function of reproductive hormones (i.e., estrogen, progesterone, testosterone) and adrenal hormones (i.e., cortisol).

*"Measuring hormone levels could tell us if a rhino is capable of reproducing," Dr. Walker said. "For example, one particular female never became pregnant. Black rhino females should be cycling every 26 days. Was she cycling? Were her adrenal hormones elevated and interfering with cycling? Maybe she could never reproduce. Knowing her hormone levels could also tell us when the female is receptive to mating so that we can introduce a male rhino at just the right time. If they become pregnant, and with a gestation of 15 to 16 months, hormone measurements can also help us determine when we can expect to have babies on the ground."*

The good news is that the hormone metabolites can be measured non-invasively in urine and feces. This is important because drawing blood requires the getting up close to the rhinos.

*"We try to do everything non-invasively, and without them knowing," Walker explains.*

Dr. Walker's group developed a tailor-made enzyme immunoassay (EIA) to measure hormone metabolites in fecal material. Dr. Walker, however, needed to separate and identify the different metabolites and to avoid cross reactions. *"We're measuring hormone metabolites in feces,"* said Dr. Walker, *"not the native hormones in blood—and we need to know exactly what we are measuring."*

## Thermo Scientific Applications Lab U.K. Steps in to Help

While touring the facility, Dr. Harald Ritchie, Thermo Scientific Director of Chromatography Business Development U.K., asked if Dr. Walker had considered using HPLC to identify and separate the metabolites of interest. *"I pretty much jumped up and down and said I would love to, but we didn't have the expertise or funds to purchase and operate a chromatography system ourselves,"* Dr. Walker recalls.

Applications scientists, Luisa Pereira and Monica Dolci at the Thermo Scientific applications laboratory in Runcorn, UK stepped in to help. They were ideally suited for this as in their everyday working life they are responsible for producing applications of Thermo Scientific chromatography consumables for customers. They also have a vital role in product development and testing which has developed their talent for applying products to solve a wide range of different customer problems. In this case, they developed a method to separate and identify the rhino hormone metabolites by HPLC using a Thermo Scientific™ Hypersil GOLD™ column on the chromatography system in an 18-minute analysis.

*"We needed to use a long run to separate other compounds in the sample,"* Luisa said. *"It was a dirty sample because of the minimal sample preparation."* Dr. Walker uses methanol to extract the hormone metabolites from fecal samples and dries this before sending to the Thermo Scientific applications laboratory. *"Separating the fractions was essential; some metabolites cross-react so each needs to be measured separately by enzyme immunoassay (EIA),"* Monica added. Fractions were sent back to the zoo for EIA analysis.

*"The Thermo Scientific applications lab provided the expertise and services we needed. They gave us a lot of scientific credibility, which enabled us to publish scientific papers. We are hugely grateful, it was beneficial to us,"* Dr. Walker added.

## Babies on the Ground

This critical thinking and forward thinking project has resulted in a baby boom at the zoo. There have been five births in recent years, with the last three being born from Nov 2012 to June 2013. Zoo officials can now predict when a female will be in season and know when to put the male and female together. After 3–4 months, they can confirm pregnancy and fast-track the male on to their next breeding partner. They can also determine when a pregnancy has been lost.

The project expanded. *"It became quite a big project as we were asked if we could expand the hormone analysis to the field,"* Dr. Walker said.

*The Thermo Scientific Applications Lab developed a method to separate and identify the rhino hormone metabolites by HPLC using a Thermo Scientific Hypersil GOLD column on the chromatography system in an 18-minute analysis.*

## SPE Cartridges Find Unique Use Helping Black Rhinos in the Wild

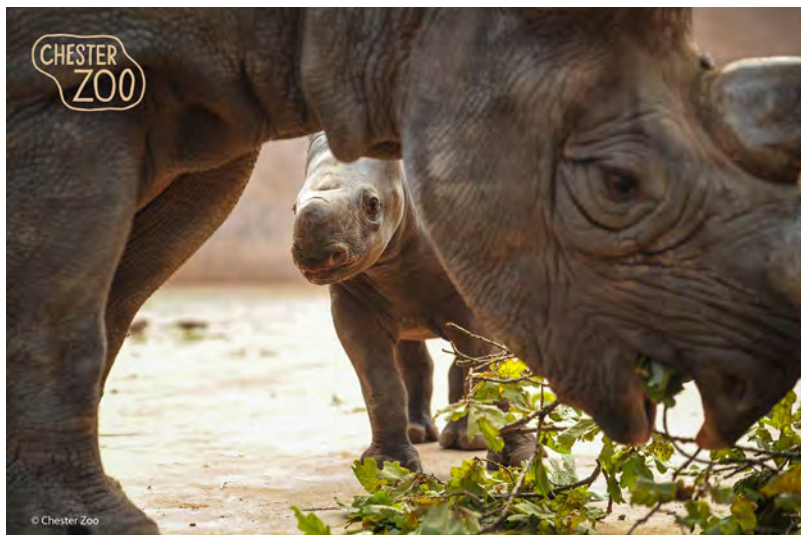
All wild black rhinos live in reserves in what's called meta-populations. When animal dung samples are collected in a zoo, keepers can pick up the sample and put it in a freezer. But what does one do in the field collecting animal dung samples for endocrine analysis? There is often no electricity on site or freezers to store the sample. That's where the solid-phase extraction comes in. Field workers can actually do a quick and effective sample extraction in the field using sample diluted with alcohol that can be loaded onto a Thermo Scientific™ Hypersep™ solid phase extraction cartridge. This preserves the sample without the need for refrigeration for up to six months allowing analysis to take place in a laboratory at a later date.

### Thermo Scientific LC-MS System in Their Future?

Dr. Walker is talking with the Thermo Scientific application lab to see if they can help with a similar project for macaques, which are primates, to investigate what hormones are responsible for aggressive behavior in males as well as how contraception can be effectively used to manage the genetic diversity of a population. *"We hope to perform hormone analyses in the future by LC-MS,"* said Harald Ritchie. *"Antibodies are limited in supply and the EIA assays can be slow and temperamental. With LC-MS, we can look at all hormones in one run. There's a real trend in wildlife to use LC-MS."*

It turns out that this experience could turn into a niche market for Thermo Fisher Scientific and give something back by helping a critically endangered species at the same time.

*"Field workers can actually do a quick and effective sample extraction in the field using sample diluted with alcohol that can be loaded onto a Thermo Scientific Hypersep solid phase extraction cartridge. This preserves the sample without the need for refrigeration"*



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