TECNIPLAST®

innovation through passion

Advanced DVC® Platform Expands Insight into Preclinical Mouse Models

The use of the Tecniplast 24/7 "smart cage" monitoring system supplies novel preclinical metrics to maximize findings and improves animal health and well-being.

Summary

Non-intrusive, automated recording of mice home-cage behaviour expands the collection and analysis of unbiased data, maximizing not only welfare but also the knowledge gained from the use of preclinical animal models. The market-leading Digital Ventilated Cage (DVC®) platform from Tecniplast is capable of continuously detecting spontaneous animal activity from the cage rack. It has contributed to the development of new metrics for experimentally documenting ALS symptoms in asymptomatic mice, replaced stressful and time-consuming behavioural analyses in stroke models, and detailed the progression of oncology therapeutics.

Introduction

Mice models are almost ubiquitous in biomedical research. They are used in more than 95% of preclinical animal studies[1] and take into consideration the complexity of the complete living organism. But these models also have intrinsic challenges and moral imperatives to maximize data acquisition. As with any experimental system, reproducibility is key.
Many factors, including the cage environment, can impact the testing response and result in inconsistency in experimental outcomes. These unplanned variables have focused attention on the development of high-density, home-cage monitoring systems for non-intrusive recording of laboratory animals' home-cage behaviour.[2]

Automated 24/7 monitoring data enable unbiased, extensive and quantitative assessments of behaviour and activity over time. When performed directly at the home-cage rack, additional advantages result including a reduction in handling that positively impacts animal welfare, and a decreased need for dedicated space and scientific staff to perform testing.[3]

In this white paper, we discuss the Tecniplast DVC® (Digital Ventilated Cage) platform, highlighting recent publications detailing novel metrics and findings.

Inside the DVC® Platform

The DVC® platform is comprised of sensors, hardware and software. The DVC® board underneath each cage position mechanically connects to an IVC (individual ventilated cage) rack, and is composed of a dozen electrodes in a grid configuration, connected to an integrated circuit that continuously measures their electrical capacitance. As capacitance is influenced by the matter present in each electrode's surrounding, measurements are affected by the presence of animals and water (Figure 1 and Figure 2).

Planar locomotor movements performed while close to an electrode induce significant capacitance changes; tracking these changes allows for activity monitoring. The collection of capacitance measurements from each electrode can be custom set. Studies have shown that the monitoring technology does not influence either animal welfare or behavioural repertoire.[4,5]

The DVC® boards are hardwired to a dedicated DVC® master computer, which provides both power and data connection. The DVC® master collects raw data coming from all cage positions, with the option of transferring them directly to a cloud-based application or a dedicated storage device for data processing. Overall, the amount of data generated per cage requires limited storage and computational capabilities.

Additional capabilities include infrared sensing to detect in-cage availability of food and water, and an RFID system to detect cage location eliminating misplaced or lost animals. The DVC® boards are protected by a special resin allowing routine sanitization and autoclaving.

Platform Comparison

Instead of a pipeline approach, the DVC® platform allows parallel data collection affecting all animals similarly. 24/7 observation enables the collection of a new set of data related to spontaneous animal activity and behaviour, which would otherwise not be observed.

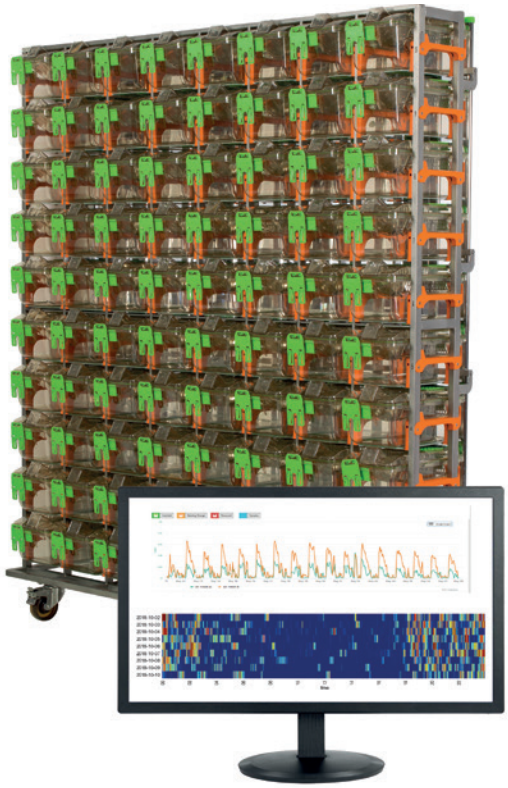

Figure 1. The DVC® platform is comprised of sensors, hardware and software to automatically monitor welfare and activity 24/7 enabling unbiased, extensive and quantitative behavioural assessments. The DVC® board underneath each cage position mechanically connects to an IVC (individual ventilated cage) rack

Several technologies exist for home-cage monitoring, ranging from video cameras, beam-breaking systems, and force transducers to other techniques based on passive infrared, piezoelectric and microwaves.[6] Each technology has trade-offs, for example, camera-based systems have the advantage of providing detailed images and the capability to observe animal locomotion and behaviours, but have limited scalability, and require computational power and mechanical set up. Other systems need ad-hoc mechanical set ups, which limits scalability and may require dedicated personnel.

DVC®-based metrics are comparable to a video camera-based tracking system with the added advantage of system scalability and reduced computational requirements.[7]

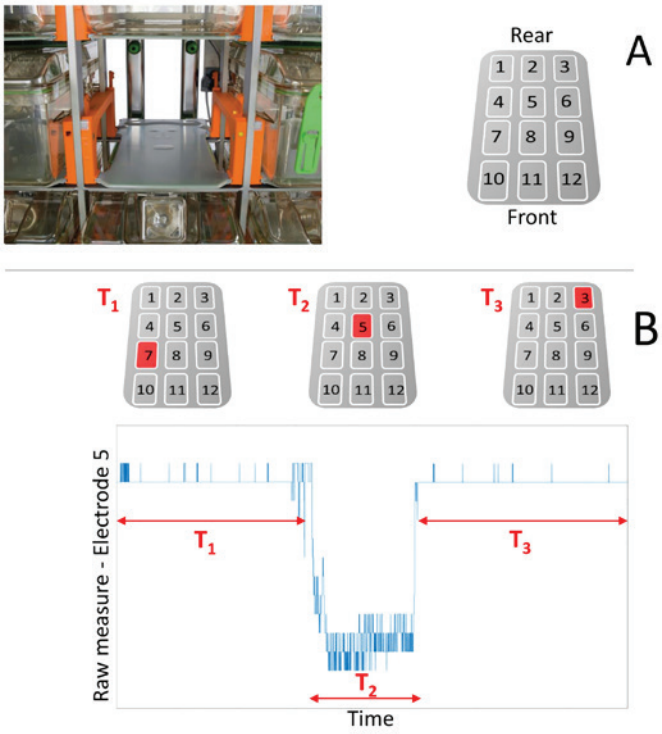

Figure 2. A. Standard IVC cage position equipped with DVC® sensing technologies. The grey board, which is coated with a special resin to allow autoclaving, is composed of a dozen electrodes in a grid configuration; the orange lateral runners contain embedded IR sensors for food and water bottle detection. B shows an example of the raw signal measured from electrode 5 when a mouse stays for T1 seconds in a cage area close to electrode 7, then moves towards and across electrode 5 during T2 seconds and then on to electrode 3 staying there for T3 seconds. [2]

Improving Animal Welfare

Several years ago, a survey undertaken by Tecniplast clearly illustrated the need for additional animal welfare monitoring, especially data focused on the cage environment to track food and water availability, automatic watering system failures, and moisture in the bedding.

"Currently 90% of cages are changed on a fixed schedule independent of the number of animals group housed," explains Giorgio Rosati, Senior Product Manager DIGILAB at Tecniplast. "The DVC® platform dramatically changes this paradigm. Cage changes are suggested daily based on real moisture in the bedding conditions." Every facility is different, but Rosati says the number of cage changes can be cut as much as 40-50% annually, thereby lowering running costs and improving animal welfare.

Every 20 minutes around the clock, the DVC® platform monitors food and water through special sensors with infrared emittance embedded into the lateral cage rack runners. When parameter thresholds are exceeded, an alarm informs animal managers to investigate and the corresponding cages can be easily located in the rack due to special on-request LED illumination.

Mice are nocturnal animals, yet rudimentary daily welfare checks take place in the daytime making it not only difficult to assess health but also cause errors due to the sheer volume of cages. The DVC® platform augments the daily check and assists in identifying critical issues; data are automatically collected during the night when the animals are more active as well as in daylight hours. Daily, a list of cages that exhibited unusual nocturnal behavioural activity can be provided.

"The DVC® platform is extremely powerful from a real-time data perspective because of its capability to continually collect unbiased and reproducible data," Rosati continues. "Each time we run an experiment we make new discoveries and then our dedicated team of data scientists, neuroscientists, and software developers works with our partners to validate, refine and implement novel meaningful measurements with new algorithms."

Studies have shown that the DVC® platform effectively identifies cages with patterns of high activity levels, signaling possible aggression incidences and providing an opportunity for early intervention.[8] The platform has also been used to continuously record activity and welfare while training mice using either food or water restriction on an appetitive operant visual discrimina-

tion task, clearly confirming the shift of the circadian rhythm triggered by the daily delivery of food and water.[9]

Reproducibility across Different Sites

Researchers compared the activity of groups of C57BL/6J mice housed in DVCs across three test sites: Consiglio Nazionale delle Ricerche (Rome, Italy), The Jackson Laboratory (Bar Harbor, USA) and Karolinska Insititutet (Stockholm, Sweden).[2] The platform detected an increase in activity preceding and peaking around lights-on followed by a decrease to a rest pattern. At lights off, activity increased substantially displaying a distinct temporal variation across this period (Figure 3).

The researchers noted that the effects of standard animal handling procedures on mouse activity were stressful and impacted in-cage activity.

Key observations were replicated across the three test sites although minor local environmental differences generated significant behavioural variances. Comparison of gender revealed differences in activity in the response to cage change lasting for days in male but not female mice. Females, but not males, showed a larger tendency for week-to-week variance in activity possibly reflecting estrous cycling.

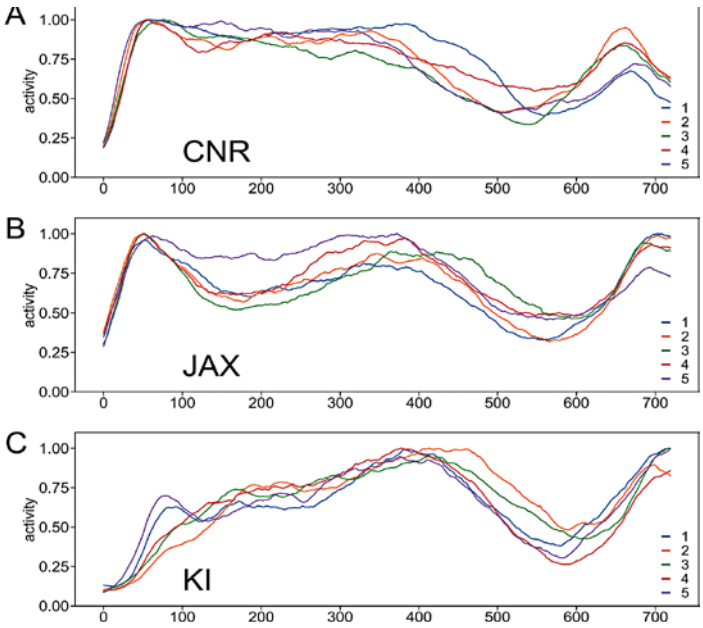

Figure 3. Average response per cage across weeks to lights-off in cages (n = 5) of female mice housed at the three sites. Traces start at light-off and end at lights on. The dark night cycle is 720 minutes. The average activity across multiple weeks is normalized to peak activity (= 1.0). There are clear differences between sites; however, the late-night pattern of activity, >400 minutes, shows less difference.[2]

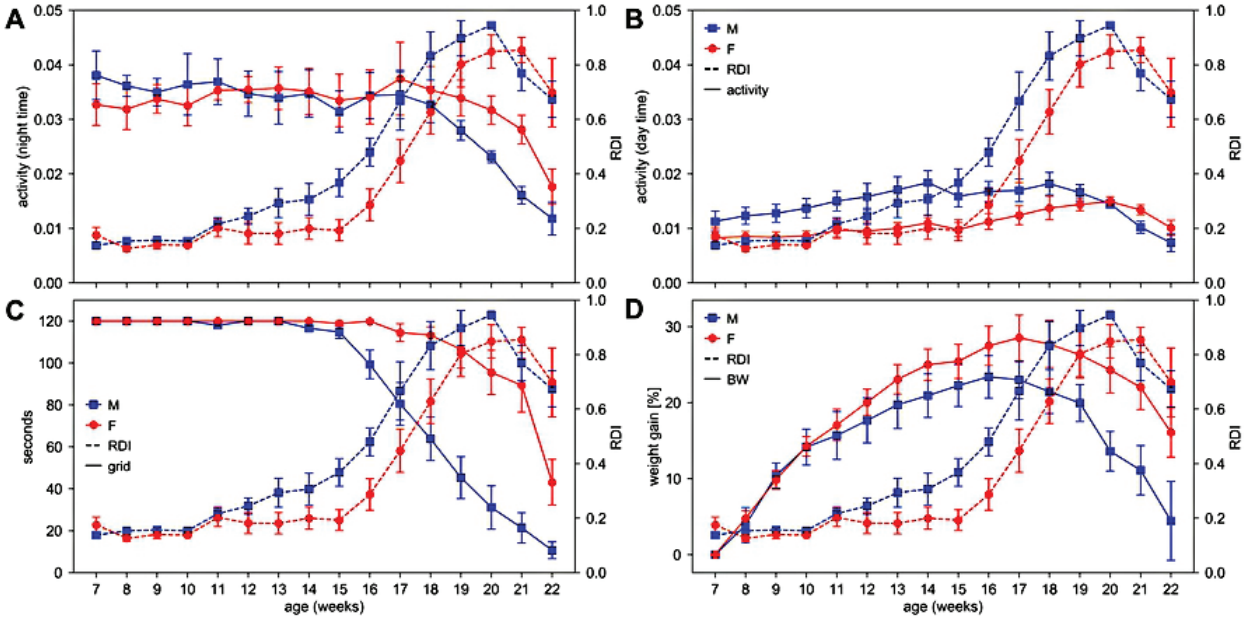

Figure 4. The DVC®-based RDI was compared with average activity during 24 hours, body weight and grid hanging tests for transgenic (TG) mice. Fig 5A compares night activity with RDI for both males and females. RDI starts increasing earlier than the start of the night activity decline. 5B shows that an increase of the RDI does not correspond to a change in the day activity. The time course of RDI and grid hanging test latency is shown in Fig 5C, where the decline in grid hanging performance appears later than when RDI starts increasing. Fig 5D shows that body weight gain peaks later than the raise of RDI.[10]

Novel Activity Metrics for ALS Monitoring

Scientists have also used the DVC® platform to capture activity patterns associating sleep and rest disturbances in a mouse model of amyotrophic lateral sclerosis (ALS).[10]

In male and female Sod1^{G93A} mice from 7-24 weeks of age, non-intrusive 24/7 long-term animal activity monitoring was assessed along with body weight decline and neuromuscular deterioration, measured by grid hanging and grip strength tests. As the neurodegenerative disease progresses, daytime activity patterns became irregular, with frequent activity bouts that were neither observed in control or younger mice.

A newly-developed digital biomarker, the Rest Disturbance Index (RDI), was used to quantitatively capture the activity pattern irregularities (Figure 4). RDI showed a high negative correlation with grid-hanging performance and body weight gain, especially in males. The findings suggest that RDI is a robust measure of ALS-related sleep fragmentation at the symptomatic stage and represents an additional marker to detect pre-symptomatic signs of disease.

In short, monitoring DVC® activity confirms the observations and expected sex differences observed in previous studies. Home-cage observation provides clear advantages to data collection, as it allows hands-off animal monitoring during resting and active periods and, crucially, gathers metrics based on objective and replicable algorithms.

Stroke Research

In another study looking at hydroxytyrosol as an acute therapeutic strategy after ischemic stroke, C57BL/6JRj mice were housed in DVC® cages to study individual locomotion before and after surgery.[11] Stroke operations were performed blind, therefore animals were housed singly to control discomfort that could arise in group-housed situations with dominant cage mates.

DVC® metrics were used to study individual mouse locomotion by calculation of activity, walked distance, walked velocity, total turns, and laterality index. This novel approach revealed decreased night-time activity in stroke mice one week after surgery. Notably, during the night time of the second post-surgery week, only stroke mice showed a left-turning preference. This latter result is in line with standard behavioural tests used in preclinical stroke studies.

"We now add in the DVC® data to supplement behavioural tests and a neuroimaging battery," said Maximilian Wiesmann, postdoctoral fellow in the Kiliaan laboratory at the Radboud University Medical Center. "Stroke symptoms are both motor and cognitive. Initially, we used the DVC® to monitor recovery after surgery, activity and occupancy – the location of the mouse in the cage – as health indicators but we wanted to include more parameters since the DVC® measurements are automatic."

The Radboud team performs behavioural tests during the day, while the mice are resting, and these novel environments induce stress. "Now we can observe their activity during their active night periods when they are not disturbed. This additional information informs stroke research because mice, similar to many animals, hide their symptoms, and an advanced behavioural testing battery is needed to detect changes in motor activity. For example, preferentialleaning is not always visible in the pole test but it is visible in the night DVC® data, which fits with the literature," says Wiesmann.

As not all behavioural tests are repeatable, the DVC® data add extra value. Currently, the researchers are working on validation of the DVC® system to replace select tests, such as the open field test or pole test, which is comparable to monitoring lateral movement with DVC® data. The open field test is a repetitive 10-minute test, whereas the DVC® platform unobtrusively collects data 24/7.

Oncology Applications

For the past two years, Pierre Lainee, head of the In Vivo Research Center at Sanofi, has assessed the relevance of the DVC® platform for animal welfare and oncology applications.

"We were interested in locomotion and activity and to use the DVC® platform to monitor welfare and the clinical status of group-housed cages without animal handling," Lainee said. "Scientists performed clinical studies during the day, such as weighing, and the DVC® platform recorded nocturnal locomotion. Overall the DVC® platform could indicate a difference in the cage."

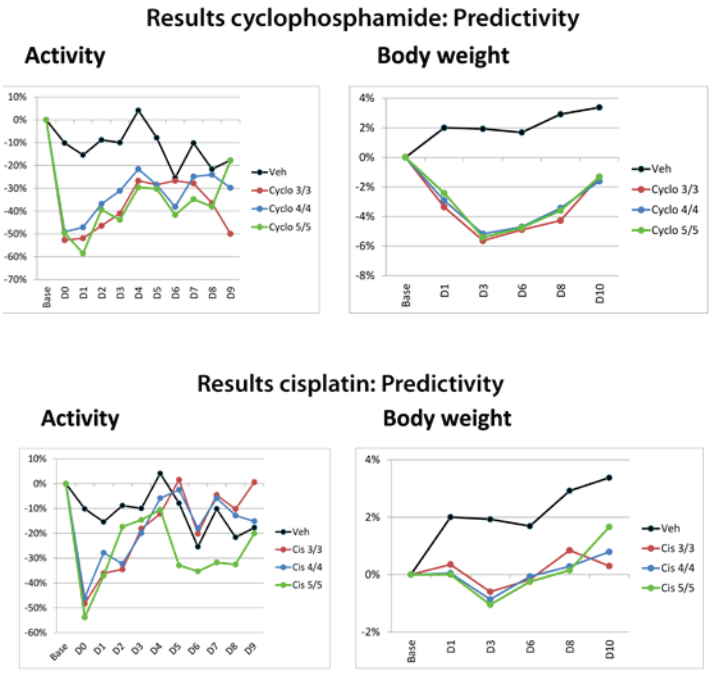

Figure 5. Two reference compounds, cyclophosphamide and cisplatin, were administered to SCID mice housed by 3, 4, or 5 animals. As expected, cyclophosphamide induced pronounced effects compared to cisplatin. Compared to controls (Veh), decreased activity was seen from the first night. All groups regardless of the number housed looked similar, and body weights correlated with activity changes. Image courtesy of Pierre Lainee, head of the In Vivo Research Center at Sanofi.

Welfare monitoring demonstrated that the frequency of cage changes could be dropped from weekly to once every 10-21 days, reducing bedding change time 30-50%, and providing better comfort. LED illumination of noted cages made the DVC® platform easy to adopt.

In a preclinical evaluation to determine if automated data collection could predict therapeutic outcomes in SCID mice, two reference compounds, cyclophosphamide and cisplatin, were administered to mice housed by 3, 4, or 5 animals. As expected, cyclophosphamide induced pronounced effects compared to cisplatin. Compared to controls, decreased activity was seen nearly from the first night. All groups regardless of the number housed looked similar, and body weights correlated with activity changes (Figure 5).

"In the past we would do nothing during the night. Using nocturnal hypoactivity monitoring we could predict the toxicity of the compound administered from the first day," adds Lainee.

Next, sensitivity was evaluated to determine if a single affected mouse could be detected in a group comprised of treated and non-treated animals. When significant effects are observed as after cyclophosphamide administration, a single affected animal could be detected in a group of three animals but not in groups of four or five. However, according to Lainee, this weak sensitivity is compensated by the availability of continuous data.

"I am confident sensitivity to detect one animal out of four can be attained by algorithm improvement, and that there is a place for DVC® in research facilities," Lainee says. Even at this early stage in the Tecniplast platform's life cycle, "our scientists trust the data, and our usage will expand and improve."

Summary

The dynamic DVC® platform has demonstrated its value in welfare monitoring as well as in the automatic detection of important experimental data in mice during their high-activity period, the night, in addition to their resting period during the daytime. As the number and depth of algorithms increases the platform will solidify its role in preclinical small animal studies.

References

1. Vandamme TF. Use of rodents as models of human diseases. *J Pharm Bioallied Sci*. 2014;6(1):2–9. doi:10.4103/0975-7406.124301
2. Pernold K, Iannello F, Low BE, et al. Towards large-scale automated cage monitoring – Diurnal rhythm and impact of interventions on in-cage activity of C57BL/6J mice recorded 24/7 with a non-disrupting capacitive-based technique. *PLOS ONE* 2019 14(2): e0211063. https://doi.org/10.1371/journal.pone.0211063
3. Iannello F. Non-intrusive high throughput automated data collection from the home cage. *Heliyon* 5 (2019) e01454. doi: 10 .1016 /j .heliyon .2019 .e01454
4. Burman O, Marsella G, Di Clemente A, Cervo L. The effect of exposure to low frequency electromagnetic fields (EMF) as an integral part of the housing system on anxiety-related behaviour, cognition and welfare in two strains of laboratory mouse. *PLOS ONE* 2018 13(5): e0197054. https://doi.org/10.1371/journal.pone.0197054
5. Recordati C, De Maglie M, Marsella G, et al. Long-Term Study on the Effects of Housing C57BL/6NCrl Mice in Cages Equipped With Wireless Technology Generating Extremely Low-Intensity Electromagnetic Fields. *Toxicol Pathol.* 2019 Jul;47(5):598-611. doi: 10.1177/0192623319852353. Epub 2019 May 22.
6. Richardson CA. The power of automated behavioural homecage technologies in characterizing disease progression in laboratory mice: A review. *Applied Animal Behavior Science* 163 (2015) 19-27
7. High Throughput Automated Data Collection from the Home Cage: Digital Ventilated Cage (DVC®), Tecniplast White Paper, SR000/ING - Rev Jan2019
8. Jareca M Giles, JM, Whitaker JW, Moy SS, and Fletcher CA. Effect of Environmental Enrichment on Aggression in BALB/cJ and BALB/cByJ Mice Monitored by Using an Automated System. *J Am Assoc Lab Anim Sci.* 2018 May; 57(3): 236–243. doi: 10.30802/AALAS-JAALAS-17-000122
9. Goltstein PM, Reinert S, Glas A, Bonhoeffer T, Hübener M. Food and water restriction lead to differential learning behaviors in a head-fixed two-choice visual discrimination task for mice. *PLOS ONE* 2018 13(9): e0204066. https://doi.org/10.1371/journal.pone.0204066
10. Golini E, Rigamonti M, Iannello F, et al. Non-invasive digital biomarker for the detection of rest disturbances in the SOD1G93A mouse model of ALS. Cold Spring Harbor Laboratory bioRxiv preprint (2019) doi: https://doi.org/10.1101/2019.12.27.889246
11. Calahorra J, Shenk J, Wielenga VH, et al. Hydroxytyrosol, the Major Phenolic Compound of Olive Oil, as an Acute Therapeutic Strategy after Ischemic Stroke. *Nutrients* 2019, 11, 2430; doi:10.3390/nu11102430

COMPANY WITH QUALITY SYSTEM CERTIFIED BY DNV GL = ISO 9001 =

COMPANY WITH ENVIRONMENTAL SYSTEM CERTIFIED BY DNV GL = ISO 14001 =

TECNIPLAST

HEADQUARTERS
ITALY | Tel. +39 0332 809711 • www.tecniplast.it • E-mail: tecnicom@tecniplast.it; venditeitalia@tecniplast.it

Sister Companies
AUSTRALIA/NEW ZEALAND | Tel. + 61 2 8845 6500 • www.tecniplast.it • E-mail: info@tecniplast.com.au
CANADA | Toll Free: +1 855.347.8718 • www.tecniplastcanada.com • E-mail: info@tecniplastcanada.com
CHINA | Tel. + 86 (0)21 50810920 • www.tecniplast.cn • E-mail: tecniplastchina@tecniplast.it
FRANCE | Tél. +33 (0)4 72 52 94 41 • www.tecniplast.fr • E-mail: info@tecniplast.fr
GERMANY | Tel. +49 (0)8805 921320 • www.tecniplast.de • E-mail: info@tecniplast.de
JAPAN | Tel. +81 (0)3 5770 5375 • www.tecniplast.it • E-mail: info@tecniplastjapan.co.jp
SWITZERLAND | Tel. +41 (0)81 750 31 40 • www.indulab.ch • Email: infos@indulab.ch
UNITED KINGDOM | Tel. + 44 (0)845 0504556 • www.tecniplast.it • E-mail: info@tecniplastuk.com
USA | Toll Free: +1 877.669.2243 • www.tecniplastusa.com • E-mail: info@tecniplastusa.com

SR000/ING - Rev Jan2019