Protecting puppies against parvovirus
The science of vaccination protocols

Section I: Introduction

Vaccination is arguably the single greatest public health achievement of all time. In companion animal practice, successful vaccination programs have seen the virtual elimination of canine distemper, infectious canine hepatitis, and feline panleukopenia, although reports of sporadic cases of these diseases should prompt continued vigilance on the part of the practitioner. Although still a major pathogen, modern vaccines have rendered canine parvovirus (CPV) a disease of the young and unvaccinated.

Given the success of vaccination in controlling disease, and the frequency with which it is performed in practice, it is easy to become complacent about vaccination protocols. A regular review of vaccination protocols is recommended in order to ensure you are providing the best protection for your patients. There are many sources of information regarding vaccination protocols, some of it conflicting, ranging from guidelines produced by various professional bodies, manufacturers recommendations, peer reviewed studies, and protocols passed down through clinic lore.

The aim of this technical bulletin is to discuss some of the science underlying vaccination for puppies, with a particular emphasis on canine parvovirus, to enable you to make informed decisions on your puppy vaccination protocol.

Section II: Vaccination protocols: The art of compromise

The challenge of designing a primary vaccination protocol for puppies is that there exists two almost diametrically opposed aims in these first few months of life. As the highest risk period for infectious disease, on one hand, it would be ideal to keep puppies completely isolated until after they have completed their primary course of vaccinations. Unfortunately this approach, inherently sensible as it is, is counter to the other aim of these first few months of ensuring puppies are exposed to as many novel stimuli and environments as possible during their critical socialisation period. At first glance it may seem
that when trying to balance these competing priorities that the danger of infectious diseases such as CPV would far outweigh the risks associated with poor socialisation. The consequences of poor socialisation however, although perhaps not as dramatic as parvovirus, may be every bit as deadly. Failure to adequately socialise dogs may result in a range of behavioral difficulties, including aggression, fear and anxiety conditions, and phobia development², which may result in dogs being surrendered and euthanased ³. Less dramatically, poor socialisation and associated behaviour problems may result in affected dogs having a reduced human-animal bond with their owners. **Weighing up the risk of infectious disease versus poor socialisation for your patients is not a simple one, and this equation will be different depending on a range of circumstances, such as the practice philosophy, and the risk and incidence of disease.** Regardless of the circumstances however, the optimal solution is to use vaccines and vaccination protocols that provide protection at the earliest possible age.

**Section III: Maternal antibodies - the good and the bad**

In all species, maternally derived antibodies (MDA) provide passive immunity for the neonate in the critical first few weeks to months of life. Depending on the species and type of placentation, the neonate acquires these antibodies via the placenta *in utero*, or via the ingestion of antibody rich colostrum immediately after birth ⁴. The endotheliochorial placenta of dogs allows limited transplacental passage of antibodies, resulting in only an estimated 5 to 10% of the final MDA titre being acquired this way ⁵. The balance is acquired via the ingestion of colostrum within the first 24 hours. Absorption of colostral antibodies takes place primarily in the ileum, and is facilitated by the inherently low proteolytic activity of the intestine at this time along with the presence of trypsin inhibitors in the colostrum ⁶. The window for the absorption of colostrum is limited, with the process rapidly declining due to maturation of enterocytes and the establishment of intestinal microflora ⁴⁻⁵. After this time the presence of antibodies in the bitches milk may still provide protection against some pathogens via a process known as lactogenic immunity, however there is essentially no further systemic absorption. Thus maternal antibody titres are at a maximum in the first 24-48 hours of life, after which they are slowly depleted through normal catabolic processes or use. This decline occurs with a half-life that is characteristic of the immunoglobin class and the pathogen, which for CPV is reported as approximately 10 days ⁶.

Maternally derived antibodies are of great benefit as they provide protection for the immunonaive neonate during
the first few weeks to months of life as they make the transition from the protected \textit{in utero} environment to face the microbial challenge of the real world. Whilst their titre remains high, the passive immunity provided by MDA is able to protect puppies from virulent CPV challenge. This level is shown schematically in Figure 1. Unfortunately this protection comes at a cost, as MDA can also bind to and prevent vaccine viruses from replicating, and thus prevent their triggering an appropriate immune response. The minimum MDA titre that is able to prevent replication of a vaccine strain of CPV is lower than the titre that can protect the pup from virulent viruses, meaning that the pups MDA titre must drop into the non-protective range before a vaccine can have a chance to work. The titre to which MDA must drop before a vaccine can successfully immunise a dog will vary depending on the type of vaccine used (Figure 2). All puppies, irrespective of their starting maternal antibody titre or the specific vaccine or vaccination protocol used, will for a time be susceptible to infection prior to being able to respond to vaccination. Although this “window of susceptibility” cannot be avoided, it can be minimized as shown in Figures 3 and 4 by choosing a high titre low passage vaccine that is able to better break through MDA (see box on page 4 and 5).

\textbf{Section IV: Vaccination protocols: One size doesn’t necessarily fit all}

When it comes to canine vaccination protocols it should be remembered that not all animals are the same: risk factors for the various diseases will vary depending on both the dog’s lifestyle and its geographical location. This concept is already well recognized, with companion animal vaccines frequently divided into core and non-core categories. Core vaccines are those recommended to be given to all animals, irrespective of their circumstances, however this does not mean that core vaccine protocols cannot, and should not be individualised. Such individualization may be more appropriate, not at the patient level, but rather at the clinic level, given that the risk of the core diseases, in particular canine parvovirus, is likely similar for all patients within a geographic region. For this reason, the core vaccination protocol in an inner-city area may justifiably be quite different than that seen in a semi-rural or rural area with a high parvovirus challenge. For the non-core diseases, such as canine cough or leptospirosis, an individual risk assessment should be made for each patient, as risk factors may vary considerably depending on patient circumstances.

Consideration of the following questions (starting on page 6) may help in optimising your vaccination protocol.

\begin{figure}
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\includegraphics[width=\textwidth]{figure3}
\caption{Wide window of susceptibility with older generation low titre high passage vaccine}
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\includegraphics[width=\textwidth]{figure4}
\caption{Narrow window of susceptibility with modern high titre low passage vaccine}
\end{figure}
Evolutionarily speaking, canine parvovirus is a relatively new viral infection of dogs, being recognized for the first time in the late 1970s following outbreaks of severe and often fatal haemorrhagic gastroenteritis in dogs, and viral myocarditis in pups. The global spread of CPV was remarkable, with an almost simultaneous recognition of this new disease by researchers in Australia, Europe, and the United States. The new virus was identified as a parvovirus and named canine parvovirus 2 (CPV-2) to differentiate it from canine parvovirus 1 (minute virus of canines) which had previously been identified. As a model of disease emergence, the origin of CPV-2 has been extensively studied. It is generally accepted that CPV emerged as a host range variant of feline panleukopenia virus (feline parvovirus) most likely via adaptation of intermediate feline parvovirus like virus of wild carnivores. The original CPV-2 differed from feline parvovirus by 6 to 7 amino acids in the capsid protein. Continued evolution of CPV in the canine population has resulted in the rapid emergence of antigenic variants which are able to replicate more effectively in dogs (Figure 5). In 1979 CPV-2a emerged, differing by 5 to 6 amino acids from CPV-2. The better adapted CPV-2a rapidly replaced CPV-2 in the dog population, such that the original CPV-2 disappeared from the wild around 1981. In 1984, CPV-2b arose from CPV-2a, differing by 2 amino acids from the latter. More recently a CPV-2c has been identified in a number of countries, but has not yet been reported in Australia, where recent data indicates that CPV-2a and CPV-2b each account for approximately 50% of cases. The nature of the disease caused by the antigenic variants is similar to that caused by the original CPV-2, although the incubation period is shorter and the disease may progress more rapidly. Unlike the original CPV-2, later antigenic variants (CPV-2a, 2b, and 2c) have an expanded host range such that they are able to infect and cause disease in cats.

Following its emergence, the first step towards the control of CPV-2 was achieved through the use of heterologous vaccines (live or inactivated feline panleukopenia/mink enteritis virus vaccines) however the protection afforded by these products was limited and unreliable. Homologous CPV vaccines, initially inactivated, and subsequently modified live, were developed within a few years of the emergence of CPV. Modified live vaccines were shown to provide the best level of protection; however early vaccines had a limited ability to overcome maternal antibodies, requiring a final dose be given at 16 to 20 weeks of age, resulting in a wide window of susceptibility. Improvements in vaccine technology, in particular the use of potentiated CPV vaccines has significantly closed that window. The two key features of potentiated vaccines that allow successful immunisation in the face of maternal antibodies is the passage level of the vaccine strain and the titre of the vaccine.

Attenuation is the name given to the process by which the virulence of an organism is reduced such that they are no longer capable of causing disease, but are...
still able to replicate, and therefore trigger an immune response. Attenuation of viruses for vaccine seed stock is commonly performed by repeatedly growing a virulent virus in cell culture, a process known as passaging (Figure 6). With each passage, mutations may arise that may result in the organism becoming attenuated (losing the ability to cause disease in the animal). In general the more a virus is passed (the higher the passage number) the more attenuated the organism becomes. Whilst excessive attenuation may appear to be the ideal situation, this is often accompanied by a loss of fitness in vivo, meaning highly passaged organisms replicate less well in the animal, are less immunogenic, and are less able to overcome maternal antibodies. In contrast, low passage vaccines are better able to replicate in the host and are able to overcome higher maternal antibody levels, with a resulting decrease in the minimum age for final vaccination.

The other feature of vaccine technology which influences their ability to overcome maternal antibodies is the titre – quite literally the number of virus particles contained in the vaccine. As might be expected, in general higher titre vaccines are better able to overcome maternal antibodies than low titre vaccines (see Figures 3 and 4).
Section V: When should I start vaccinating?

Ideally the vaccination protocol should begin as soon as possible, bearing in mind the registered label claims of the vaccine (for Australian vaccines this is either 6 or 8 weeks of age). Under normal circumstances there is little requirement to start earlier than 6 weeks of age, however in outbreak situations, in pups born to known seronegative bitches, or in colostrum deprived pups, the off-label use of the vaccines in younger pups has been recommended by some. Although there is continued maturation of the immune system in the first 6 to 12 months of life, vaccination studies in pups as young as 1 day of age have shown them to be immunocompetent and capable of mounting an appropriate response to vaccine antigens.

In extremely young pups (< 4 weeks of age) there is a theoretical risk of attenuated CPV vaccine strains inducing myocarditis, and thus the risks / benefits of this approach should be carefully considered. Alternatively, in these circumstances, a killed parvovirus may be used, however these require multiple doses and are less able to overcome maternal antibodies. Advice should be sought from the manufacturer on the use of any vaccine in a pup at an age younger than the registered starting age.

Section VI: How many doses do I need?

Due to the replication of vaccine organisms after inoculation, modified live virus (MLV) canine core vaccines provide a prolonged period of antigenic stimulus, triggering both a primary and anamnestic response (Figure 7). Thus, in an immunocompetent dog in the absence of MDA, only a single dose of a core canine vaccine is required to stimulate adequate immunity. This means for an adult dog presenting for the first time, or with an unknown vaccination history, only a single dose is required to provide protection.

So what about puppies, how many doses do they need? The answer here is that technically they only need one MLV CPV vaccine that works to be protected. A course of vaccinations is however recommended for puppies due to the risk of interference from MDA and the desire to ensure protection at the earliest opportunity.

As previously discussed the MDA titre of pups can vary considerably, both between litters and even within a litter (Figure 8). If it were possible to obtain an individual pup’s maternal antibody titre for each pathogen shortly after birth, the optimum age for vaccination could be individualized on a patient by patient basis, and just a single vaccine given, however this approach is clearly not practical. Instead pups are given a series of vaccinations throughout the period in which their maternal antibody titre is likely to decrease to the point at which they can successfully respond, ensuring that the final vaccination is given after the age at which MDA are unlikely to interfere even in the pups that acquired extremely high MDA titres. For any given antigen, only one of the vaccines given in this primary course will be of benefit: those given prior to MDA decreasing sufficiently will be blocked, as will those given after the pup has already mounted an adaptive immune response to a previous vaccine. Vaccination with a modified live CPV vaccine is essentially an all-or-nothing phenomenon, and there is unlikely to be any incremental increase in the magnitude of the immune response with successive vaccinations during this primary course (for this reason the multiple core vaccines given to puppies should not technically be called boosters).

Section VII: What is the best interval to have between vaccinations?

Vaccination protocols should aim to allow a pup the opportunity to respond to vaccination at the earliest opportunity after MDA has decreased below the critical level, thereby closing the “window of susceptibility” as far as possible. This is achieved by decreasing the interval between successive vaccinations, however it is recommended that vaccines of any type are not given closer than two weeks apart. The reason for this...
minimum interval is that the non-specific immune response to a vaccine, comprising various cytokines such as interferon, may inhibit the response to subsequent vaccines. For the same reason, it is recommended not to vaccinate within 2 weeks of recovery from any infectious disease.

Whilst there is a definite minimum interval, there is no maximum inter-vaccination interval defined for MLV core canine vaccines. This is in contrast to inactivated vaccines, where the first priming vaccine must be followed relatively quickly with a booster to trigger an appropriate anamnestic response (Figure 7). Instead the maximum vaccination interval should be based on consideration of the risk of exposure to virulent CPV during the primary vaccination course. Thus for pups born into a known challenge environment (e.g. a breeder with a history of CPV on their property) minimising the inter-vaccination interval is strongly recommended.

Section VIII: When should I give the last puppy vaccine?

The timing of the final vaccination of a puppy course is one of the most controversial issues in companion animal vaccinology, in part because there is often conflicting sources of information. Both the World Small Animal Veterinary Association (WSAVA) Vaccination Guidelines and the American Animal Hospital Association (AAHA) Guidelines both recommend a final vaccination be given at 14 to 16 weeks of age. In contrast the manufacturer’s guidelines for some high titre low passage CPV vaccine such as Protech® and Duramune Adult® support an earlier finish, with final vaccination from 10 weeks of age. Such claims are based on clinical challenge trials conducted in varying pups with varying MDA titres, examples of which are as shown on pages 8 and 9. So who is correct, and what is the best age for final vaccination?

Unfortunately there is no single “correct” answer for this, and both approaches have their pros and cons. Considering only the risk of infectious disease, a later finish is desirable as this minimises the chance of a pup failing to respond to vaccination through MDA interference. This is clearly a good thing, particularly in areas with a high parvovirus challenge, not because the risk of interfering MDA is necessarily higher in these areas, but rather because the consequences of a failed vaccination are greater given that unprotected dogs are very likely to be exposed to virulent parvovirus due to its ubiquitous presence in the environment in these high challenge areas. The downside of a late finish protocol, if as is often the case, it is accompanied by advice to keep puppies isolated for a week or more after their final vaccination, is that it may significantly compromise socialisation. In addition, having a later finish, will necessarily mean, if the number of doses in the primary course is kept constant, that the interval between each vaccine will increase, resulting in a greater potential window of susceptibility.

Early finish protocols have the advantage of allowing for early socialisation of pups, however it is possible with such a protocol that a pup with exceptionally high MDA titre may not respond to vaccination. Whilst this is clearly not desirable, in areas of low parvovirus challenge, the consequence of a pup failing to respond may be small, with little risk of infection, and thus the benefits may outweigh this potential small risk.

Even if circumstances dictate a later finish, the use of an early finish vaccine still provides significant benefits for your patients by ensuring they are protected at the earliest possible time. This is also beneficial if a client does not comply with the recommended protocol and fails to return the pup for its final vaccination. Using this approach also allows for a useful compromise in higher risk environments to maximise protection and to minimise potential future behavioural problems by allowing pups to socialize more widely prior to finishing their vaccination course. In this scenario, using a potentiated vaccine such as Protech® or Duramune Adult® pups can be allowed to socialize 7 to 10 days after their 10 week vaccination, as it is known that the vast majority of pups will be protected at this time. A final vaccine at 14 to 16 weeks can still be recommended to provide protection in the rare circumstance that a pup does not respond at 10 weeks of age.

Maternally derived antibody titres can vary within and between litters. Overall in a population of pups, MDA titres will approximate a normal distribution.
To demonstrate protection against CPV after a final vaccination at 10 weeks, 14 pups with varying levels of maternally derived antibodies (MDA) were vaccinated at 6 and 10 weeks of age with Protech® vaccine. At 12-14 weeks of age the pups were challenged with a high CPV challenge (CPV-2a and CPV-2b).

**Study conclusion**

The Australian registered Protech® vaccine protects pups vaccinated at 6 and 10 weeks from a virulent challenge with CPV at 12-14 weeks.

**Vaccinates**

- Some dogs showed mild and transient signs
- In general all vaccinates were happy and healthy

**Controls**

- All sick and weak with intermediate to severe clinical signs
- Bloody/watery stools, lethargy and depression
- Death

*Clinical scores assigned based on severity of clinical signs*

**Canine parvovirus (CPV) Scores**

![Graph of Canine parvovirus (CPV) Scores](image)

> **Figure 9**

Clinical scores after CPV challenge at 12-14 weeks.
Study conclusion

The Australian registered Protech® vaccine protects pups vaccinated at 8 and 10 weeks from a virulent challenge with CDV and CPV at 12 weeks.

In another study, puppies with varying levels of maternal antibodies were vaccinated at 8 and 10 weeks with Protech® vaccine. These pups were challenged at 12 weeks of age with virulent canine distemper virus (CDV) and virulent canine parvovirus (CPV-2a and CPV-2b).

**Canine distemper virus (CDV)**

Clinical signs 1-21 days post challenge in vaccinates were not present.

Clinical signs 1-21 days post challenge in control dogs included:
- Depression/lethargy
- Mild-severe ocular and nasal discharge
- Dehydration and inappetance
- Death

![Figure 10](image_url)  
Clinical scores in vaccinates & controls after CDV challenge.

**Canine parvovirus (CPV)**

Clinical signs 1-14 days post challenge in vaccinates were mild and transient.

Clinical signs 1-14 days post challenge in control dogs included:
- Vomiting
- Watery/mucoid/bloody stool
- Lethargy/depression/dehydration
- Death

![Figure 11](image_url)  
Clinical scores in vaccinates & controls after CPV challenge.
Section IX: How long after vaccination before the pup is protected?

This is a common question from new owners keen to take their latest family addition out and about. In the absence of interfering levels of MDA, there is a rapid onset of immunity following vaccination with modified live virus core vaccines. The speed of onset is antigen dependent. Immunity to CPV may develop as soon as three days after vaccination, and is usually present within 5 days, with immunity to canine distemper occurring more rapidly, and canine adenovirus being a little slower. To take into account individual animal variation, reasonable standard advice would be 7 to 10 days after vaccination.

Section X: Do I need to alter my vaccination protocol for specific breeds?

Whether certain breeds truly have an increased risk of canine parvovirus infection is a difficult question to answer. Apparent increases in incidence in certain breeds may simply reflect increased breed popularity in some areas, coupled with a greater challenge and lower vaccination rates rather than true breed susceptibility. Historically certain breeds, including Rottweilers and Dobermans, have been considered to be more susceptible to CPV infection, resulting in recommendations for altered vaccination protocols, including a later finishing age. These recommendations have been based on the findings of a number of early studies showing an increased risk for these particular breeds, in addition to anecdotal observations from practicing veterinarians. A reduced ability of these breeds to respond to parvovirus vaccination has been suggested to account for their increased incidence; however studies have been conducted which have failed to show a difference in the immune response to CPV vaccination in these breeds. It is known that a small percentage of dogs may be genetic non-responders to vaccination, failing to respond to particular antigens despite repeated vaccination in the absence of interfering MDA. Thus there remains the possibility that certain breeds, or more likely breed lines, may mount sub-optimal immune responses to particular antigens, however there is currently no solid scientific data supporting the requirement for altered vaccination protocols for any breed in relation to canine parvovirus. If there is a concern that a particular breed line may be associated with poor response to vaccination, antibody titres measured 3 to 4 weeks post vaccination can be used to determine whether a particular animal has responded appropriately.

Section XI: How do I interpret a positive parvovirus test after vaccination?

Gastrointestinal disease in young puppies is a frequent presentation. In many cases this is mild and requires symptomatic treatment only, however in cases of severe disease, particularly in high challenge environments it is important to consider parvovirus infection. It has long been considered that recent vaccination with modified live CPV vaccines creates a diagnostic dilemma due to the risk of false positive test results from the detection of shed vaccine organisms, as commercially available faecal antigen or PCR tests are unable to differentiate between vaccine strains and virulent field viruses. Several studies have looked at the likelihood of false positives arising from vaccine strains in recently vaccinated dogs. In the first study, none of 64 seronegative pups vaccinated with one of six different modified live CPV-2 vaccines tested positive for...
parvovirus antigen using an antigen test. Faecal shedding of the vaccine strain was confirmed in approximately one third of these dogs using a more sensitive haemagglutination assay. More recently a second study has looked at viraemia and faecal shedding post vaccination with CPV-2 and CPV-2b vaccines. This study confirmed the previous findings in that although vaccine virus could be detected by PCR, there was no diagnostic interference with faecal antigen testing. Thus, while it is theoretically possible that a vaccine virus shed by a recent vaccinee may be detected by an in clinic faecal antigen test, the results of available studies suggest this an unlikely occurrence. From this it is reasonable to conclude that a pup with a positive faecal parvovirus antigen test and compatible clinical signs should be considered to be infected with a virulent virus and treated accordingly. In these circumstances, given the incubation period of CPV is reported from 4 to 14 days (although 4 to 7 days is more typical) the infected pup may have been exposed and infected up to two weeks prior to vaccination, or at or shortly after the time of vaccination, but prior to the development of full immunity.

Section XII: Why might a pup fail to respond to vaccination?

When used with appropriate protocols, modern vaccines are highly effective at providing immunity against canine parvovirus. Rarely an animal may fail to respond to standard vaccination protocols. There are a number of factors which may impact the effectiveness of the immune response following vaccination (Figure 13). Broadly speaking these may be divided into factors relating to the vaccine and those relating to the animal.

Vaccine factors

Modern vaccines are produced to the highest standards of manufacturing making batch related problems unlikely. Incorrect storage or administration of vaccines may however reduce their effectiveness as chemical or physical insults can reduce the titre of the live vaccine organisms, resulting in a sub-immunising dose being given. Increased temperature is the enemy of live vaccines, and it is important that vaccines be stored and used according to the manufacturer’s recommendations.

Animal factors

- Maternally derived antibodies – Interference by maternal antibodies is the most common reason a puppy will fail to respond to vaccination. Strategies to minimise this have been discussed in the preceding pages.
- Genetic non-responders – These are estimated to occur at a rate of 1 in 1000 for canine parvovirus and 1 in 5000 for canine distemper. Genetic non-responders lack specific lymphocyte subsets capable of recognizing a particular antigen, and thus can be vaccinated multiple times and fail to mount a protective immune response. The overall prevalence of genetic non-responders may have decreased over time due to the effects of natural selection eliminating such dogs from the breeding pool.
- Concurrent illness – Any concurrent illness may result in a dog mounting a sub-optimal immune response. Veterinarians should ensure dogs are systemically well with a history and physical examination prior to vaccination.
- Medication – Vaccination with modified live vaccines should not be performed in animals undergoing treatment with immunosuppressive drugs. Vaccination of immunosuppressed dogs may result in a sub-optimal immune response, and rarely with the induction of vaccine associated disease due to a reversion to virulence.
Section XIII: Conclusion

Parvovirus is, and will remain, a significant threat to Australian dogs due to a significant population of unvaccinated, and therefore susceptible hosts, and its high environmental stability. When considering a vaccination protocol to protect puppies it is important to consider both the risk of parvovirus infection, during and after the vaccination course, and also the potential risk associated with impaired socialisation to determine the optimal protocol for your patients. The relative importance of each of these may vary, resulting in sometimes considerably disparate protocols between clinics and locations. Whilst there are some definite things to avoid in regards to puppy vaccination protocols, if the relative risks are assessed, and the underlying science of vaccinology and various practical factors considered, a number of effective protocols can be established. Doing this will ensure pups are provided the highest level of protection at the earliest possible time.

Summary

- There is no one size fits all vaccination protocol
- Vaccination protocols should be designed considering the risks of infection and the risks associated with limited socialisation
- Interference by maternal antibodies is the major cause of vaccine failure in pups and is the reason for the requirement for a course of multiple vaccines
- Vaccine choice and vaccine protocols should aim to minimize the window of susceptibility and provide protection at the earliest possible age

References:
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20. Boehringer Ingelheim Pty Limited ABN 52 000 452 308. Animal Health Division, 78 Waterloo Road, North Ryde NSW 2113. Toll free: 1800 038 037. BIB197A-08/14

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