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Veterinary Medicines Division

Committee for Veterinary Medicinal Products (CVMP)

CVMP assessment report for a worksharing variation requiring assessment for CircoMax and CircoMax Myco (EMEA/V/C/WS2429)

Vaccines common name: Porcine circovirus vaccine (inactivated recombinant), Porcine circovirus vaccine (inactivated, recombinant) and Mycoplasma hyopneumoniae vaccine (inactivated)

Assessment report as adopted by the CVMP with all information of a commercially confidential nature deleted.

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1. Introduction

1.1. Submission of the variation application

In accordance with Article 62 of Regulation (EU) 2019/6, the marketing authorisation holder, Zoetis Belgium SA (the applicant), submitted to the European Medicines Agency (the Agency) on 1 February 2023 an application for a variation requiring assessment for CircoMax and CircoMax Myco, following a worksharing procedure.

1.2. Scope of the variation

Variation(s) requested	
G.I.4	G.I.4 - Change(s) in the Summary of Product Characteristics, Labelling or Package Leaflet due to new quality, preclinical, clinical or pharmacovigilance data.

The variation is to add the option of administering CircoMax Myco and CircoMax intramuscularly, using needle-free devices. Additionally, some editorial changes to the SPC/PI are proposed; the name of the marketing authorisation holder is corrected from Zoetis Belgium S.A. to Zoetis Belgium.

1.3. Changes to the dossier held by the European Medicines Agency

This application relates to the following sections of the current dossier held by the Agency:

Part 1, Part 3 and Part 4

1.4. Scientific advice

Not applicable.

1.5. Limited market status

Not applicable.

2. Assessment

Safety

No specific safety studies were presented initially, but safety-relevant observations were made in the dye studies and in the clinical study by Cho et al. (2022).

Five supportive published studies were included in section 3b Preclinical of the dossier. These studies were included as arguments for additional safety benefits from the use of needle-free devices but did not relate specifically to CircoMax and CircoMax Myco.

Baker SR, Mondaca E, Polson D, et al. Evaluation of a needle-free injection device to prevent hematogenous transmission of porcine reproductive and respiratory syndrome virus.] Swine Health Prod. 2012;20(3):123-128. (p3b-baker-et-al-2012). This study was carried out with a group of PRRSV-inoculated pigs which were then vaccinated with a *Mycoplasma hyopneumoniae* vaccine (group 1) using the traditional needle-syringe system. When the same needle-syringe was used to vaccinate PRRSV-free pigs (group 2), all pigs eventually tested positive for PRRSV RNA, whereas when a needle-free device was used first in group 1 and subsequently in a group of PRRSV-free pigs (group 3), three pigs out of twelve became PRRSV positive. The authors concluded the use of the needle-free device reduced but did not prevent hematogenous transmission of PRRSV.

Chase CCL, Daniels CS, Garcia R, et al. Needle-free injection technology in swine: Progress toward vaccine efficacy and pork quality. J Swine Health Prod. 2008;16(5):254–261 (p3b-chase-et-al-2008). This is a review paper on the use of needle-free injection devices, focussing on transdermal application. The authors conclude that there is an increased interest in vaccination with needle-free devices in swine due to two main factors:

- immunology research, indicating that targeting dendritic cells in the skin and the subcutaneous tissues results in improved immune response with minimal antigen doses, and
- implementation of pork quality assurance standards to minimise needle-site lesions that are the result of broken needles, bacterial contamination, or both.

Hemsworth and Coleman (2011) The Stockperson and the Productivity and Welfare of Intensively Farmed Animals (204 p). This book is general introduction to the ways farm personnel can aid in improving animal welfare.

Keenliside, Wilkinson, Poiron, Schmidt, Goller and Willis (2005) Comparison of injection sites between needle-free injections and needle injections in swine (p3b-keenliside-et-al-2005). This study examined drug placement with the needle-free injection (NFI) with four sizes of pig. One pig of each size category (nursing piglet, nursery pig, market hog, and sow) was injected with blue dye using the NFI and conventional needles. Animals were euthanised within 30 minutes and injection sites examined by dissection. The NFI delivered dye intramuscularly (IM) in both nursing and nursery pigs. Dye was well dispersed through the skin, subcutaneous (SQ) and muscle layers. Needle injections in the nursing and nursery pigs produced more tissue damage and deposited dye in a bolus deep in the muscle. In the sow and market hog, the NFI placed dye intradermally (ID) and subcutaneously (SQ) only. Thicker skin and fat in older pigs appeared to prevent IM placement.

Paquin, Achacha, Toburen (2005) The first effective transdermal, needle-free injection in Canada (p3b-paquin-et-al-2005). Pigs were vaccinated against *Mycoplasma hyopneumoniae* and PRRS via needle-syringe or a needle-free device. It was concluded that blood samples collected for up to 42 days post-injection of the vaccinated pigs showed that both injection methods produced similar serological responses.

Four studies were included in part 3c:

Daniels and Funk (2009) Prevalence of carcass defects in sows at harvest (p3c-daniels and funk-2009). In this study, observation focused on identifying carcass lesions in areas of potential injection (neck, hip, shoulder, perivulvar, ham, other). Of all sow carcasses (N=3200), 84.9% had no lesions, 11.2% had a neck lesion, 2.7% had a lesion on the hip, 0.3% on the ham, 0.6% in the perivulvar region, 0.06% on the loin, 0.6% on the shoulder, and 0.5% of sow carcasses had 2 lesions. The study identified a large prevalence of injuries which can be associated with injections, whereas the precise cause (physical damage, infection or injection site reaction to the product) of the damage was not detailed.

Ko EY, Cho J, Cho JH, Jo K, Lee SH, Chung YJ, Jung S. Reduction in Lesion Incidence in Pork Carcass Using Transdermal Needle-free Injection of Foot-and-Mouth Disease Vaccine. Korean J Food Sci Anim Resour. 2018 Dec;38(6):1155-1159. doi: 10.5851/kosfa.2018.e46. (p3c-ko-et-al-2018). A total of 983 growing pigs were

vaccinated via needle-syringe or a needle-free transdermal device. The pigs were slaughtered at market weight and examined for injection site lesions. Pigs vaccinated via needle-syringe had an incidence of lesions of 19.17% whereas the pigs vaccinated with the needle-free device showed 4.35% lesions. It is noted that the needle-free application was transdermal, whereas the needle-syringe application was intramuscular.

Royer and Milward (2006) Efficacy and safety of needle-free transdermal delivery of a novel Mycoplasma hyopneumoniae bacterin (p3c-royer-et-al-2006). The study showed that vaccinated pigs had higher antibody responses (ELISA) than unvaccinated and a lower percentage of pneumonic tissue after challenge. During the field safety study, no systemic adverse events associated with vaccination were observed. Palpable injection site swellings were typical of adjuvanted bacterins and dropped to negligible levels by day 35 post-vaccination.

Temple, D., Escribano, D., Jiménez, M. et al. Effect of the needle-free "intra dermal application of liquids" vaccination on the welfare of pregnant sows. Porc Health Manag 3, 9 (2017). https://doi.org/10.1186/s40813-017-0056-3 (P3c-temple-et-al-2017). The study compared different welfare parameters between pregnant sows vaccinated with a needle-free device and sows vaccinated with needle-syringe. The study showed benefits in favor of the needle-free application with regard to a lower frequency of high pitch vocalisations, retreat attempts and turning back. General activity level after vaccination was also better among the needle-free sows. The sows were also examined for a number of physiological parameters: At 48 hours post-vaccination, needle-free sows tended to have lower blood C-reactive protein levels compared to needle-syringe vaccinated sows. Blood haptoglobin levels did not differ significantly between treatments 48 hours post-vaccination. Chromogranin A tended to show a lower increase after the needle-free application, whereas salivary alpha-amylase and salivary cortisol did not differ between treatments when measured 25 minutes post-vaccination. The authors concluded that that needle-free intradermal vaccination is a promising strategy to reduce fear and pain reaction of gestating sows during vaccination.

The applicant conducted a new GLP safety study (B924N-ES-23-C32) to evaluate the safety of the repeated administration of a single dose of 1 ml in the right neck of CircoMax Myco administered intramuscularly to 2-day-old piglets and revaccinated 3 weeks later with 2 ml in the left side of the neck using a needle free injection device (NFID, brand name Pulse FX). Hence, ten animals (T02 group) were administered CircoMax Myco via NFID and another 10 animals with PBS via the same method (T01). The operating pressure was set to 60 - 63 psi for the first vaccination (the nozzle size was 0.27 mm) and to 95 - 100 psi for the second vaccination (nozzle size 0.35 mm).

Increases in rectal temperatures after the two vaccinations were within the range already described in the SPC.

Transient local reactions (palpable swellings up to the 2-5 cm category, redness at the injection site) at the injection site were observed after the first vaccination in all (9 out of 9) of the vaccinates (T02), and in 66.7% (6 out of 9) of the control group (T01). In addition, hematomas at the injection site were observed in both T01 and T02 group animals, following first vaccination. These hematomas, which all resolved before D4, were suggested to be caused by the high pressure applied by the NFID to administer the 1 mL dose volume and the thin skin in neonate piglets.

After the second vaccination (2 mL dose), local reactions (small palpable swellings, redness) were observed in all (9 out of 9) vaccinates and in 80% (8 out of 10) of the control (T01) group. No hematomas in the injection site were observed after the second vaccination.

Efficacy

Pre-clinical studies

A series of studies were performed to evaluate the depth, distribution, and consistency of delivery of a liquid dose (0.5 ml - 2.0 ml) of a methylene blue dye solution blended with 10% "SP oil" when delivered via conventional needle (T01) versus delivery via needle-free device (T02 – Pulse FX device) into the neck muscle of pigs. The liquid dye formulation was blended with 10% SP Oil to mimic the viscosity common to several Zoetis swine inactivated vaccines.

The studies were:

Evaluation of the Deposition of 0.5 ml Methylene Blue Dye Administered in the Neck Muscle of Neonatal Pigs via a Needle-Free Injection Device (B920R-US-22-B80)

Evaluation of the Deposition of 1.0 ml Methylene Blue Dye Administered in the Neck Muscle of 21 Day of Age Pigs via a Needle-Free Injection Device (B920R-US-22-B81)

Evaluation of the Deposition of 2.0 ml Methylene Blue Dye Administered in the Neck Muscle of 21 Day of Age Pigs via a Needle-Free Injection Device (B920R-US-22-B82)

The needle-free device in these studies was Pulse FX device. Pressure settings of the needle-free device was determined by manufacturer instructions based on pig age and dose volume; needle gauge size was selected based on recommendations for pig age and size.

Separate studies were conducted for neonates (p4b-study-b920r-us-22-b80, 1-3 days of age, 0.5 ml dose) and nursery pigs (p4b-study-b920r-us-22-b81, 3 weeks of age, 1 ml dose; p4b-study-b920r-us-22-b82, 3 weeks of age, 2 ml dose) with a minimum of 30 pigs per treatment enrolled in each study. Each animal received one injection via needle-syringe application on one randomly assigned side of the neck, and the same dose given via the needle-free device on the other side of the neck. Immediately following the injections, each animal was humanely euthanised, and necropsy of the injection area was performed. Injection depth was measured and percent distribution of dye into the dermis, subcutis, and muscle was recorded.

Statistical evaluation

Percentage of dye deposited in the muscle was transformed with an arcsine square root transformation prior to statistical analysis. Treatment T02 was considered non-inferior (NI) to T01 if the lower 90% confidence limit of the difference between treatments (T02-T01) was *to the right of* -20%.

Results

Results from all 3 studies confirmed that intramuscular injection via the needle-free device is non-inferior to intramuscular injection via needle and syringe (Table 4 below).

Dye Study 1 (B920R-US-22-B80, 0.5 ml, neonatal pigs) results showed that injections given via the needle-free device and via needle-syringe both resulted in a back-transformed mean of 100% of dye being deposited into the muscle. The lower non-inferiority value was calculated as -2.48%, which is *to the right of* the pre-determined non-inferiority margin of -20%, thereby confirming that intramuscular injection of a 0.5 ml dose delivered to neonatal pigs via a needle-free device is non-inferior to intramuscular injection via needle and syringe.

Dye Study 2 (B920R-US-22-B81, 1 ml dose, 3-week-old pigs) results demonstrated non-inferiority when administering a 1 ml dose intramuscularly with a needle-free device versus needle syringe based on the non-inferiority value of -12.01%. The needle-free injection device deposited a mean of 98.1% of dye into the neck muscle of a 3-week-old pig, and the needle deposited 100% of the dye into the neck muscle.

Dye Study 3 (B920R-US-22-B82) results demonstrated that the 2 ml dye dose via the needle-free injection device deposited a mean of 95.6% of dye into the neck muscle of a 3-week-old pig and the needle deposited 100% of the dye into the neck muscle of a 3-week-old pig. The lower non-inferiority limit was determined as -19.52% which is close to, but not passing, the non-inferiority margin.

Table 4. Dye Deposition Studies Summary Results

Study ID	Dose (ml)	Animal age	Percent deposited in Muscle (back transformed least square mean)		Lower non-Inferiority limit (%)
			Needle	Needle Free	
			T01	T02	
B920R-US-22-B80	0.5	1–3 days	100	99.96	-2.48
B920R-US-22-B81	1.0	3 weeks	100	98.1	-12.01
B920R-US-22-B82	2.0	3 weeks	100	95.6	-19.52

Conclusions

In conclusion, intramuscular injection by a needle-free device was non-inferior to injection given intramuscularly with a needle and syringe as demonstrated by non-inferiority values of -2.48%, -12.01% and -19.52% for 0.5 ml, 1 ml and 2 ml doses respectively.

Additional data was provided on injection depth in the three studies:

Study number	**Age of piglets**	**Injected volume**	**Depth mean (mm)**		**P values**
			Needle and Syringe	**Needle Free Injection Device (NFID)**	
B920R-US-22-B80	1-3 days old	0.5 mL	21.2	23.3	P=0.0502
B920R-US-22-B81	3 weeks old	1 mL	37.9	31.8	P=0.0041
B920R-US-22-B82	3 weeks old	2 mL	39.8	37.8	P=0.3401

The new analysis of injection depth showed that the difference in penetration of the vaccine between application method is limited. In the 3-week-old pigs, the dye solution penetrated deepest into the muscular tissues when given via syringe needle. In 1-3-day-old piglets, the dye solution penetrated numerically deeper (but not significantly deeper (23.3 mm versus 21.2 mm, P=0.0502) when given via the needle-free device. This latter result is still with the 0.5 ml dose and the results could be different with the 1.0 ml dose.

It can be concluded that the studies with dye solutions can be considered representative of injection with CircoMax and CircoMax Myco since the amount present on the skin surface was negligible and since the physicochemical characteristics were sufficiently similar to those of the vaccine.

Clinical studies

One published study from a peer-reviewed journal is presented in representation of clinical studies.

Cho H, Ahn Y, Oh T, Suh J, Chae C. **Non-Inferiority Field Study Comparing the Administrations by Conventional Needle-Syringe and Needle-Free Injectors of a Trivalent Vaccine Containing Porcine Circovirus Types 2a/2b and Mycoplasma hyopneumoniae.** Vaccines (Basel). 2022 Feb 24;10(3):358. doi: 10.3390/vaccines10030358. PMID: 35334988; PMCID: PMC8952852.

Experimental design:

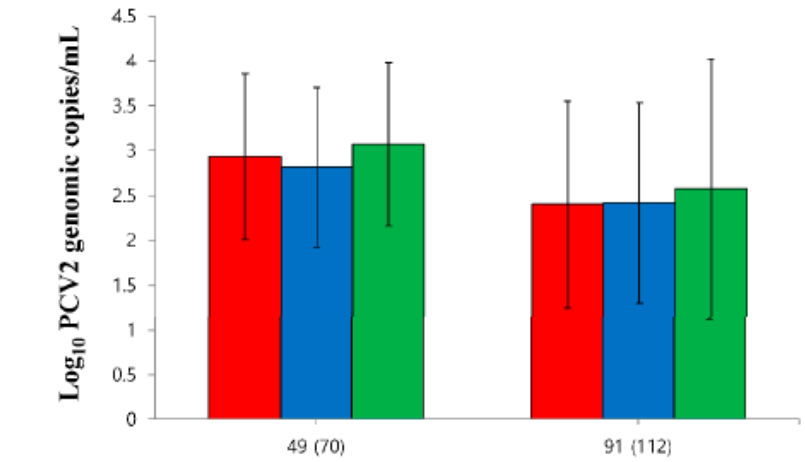
The experiment was performed on a farm with a clinical history of subclinical PCV2 infection and enzootic pneumonia. The farm was seropositive for PRRS but clinical signs were absent. The sows on the farm were Large White × Landrace.

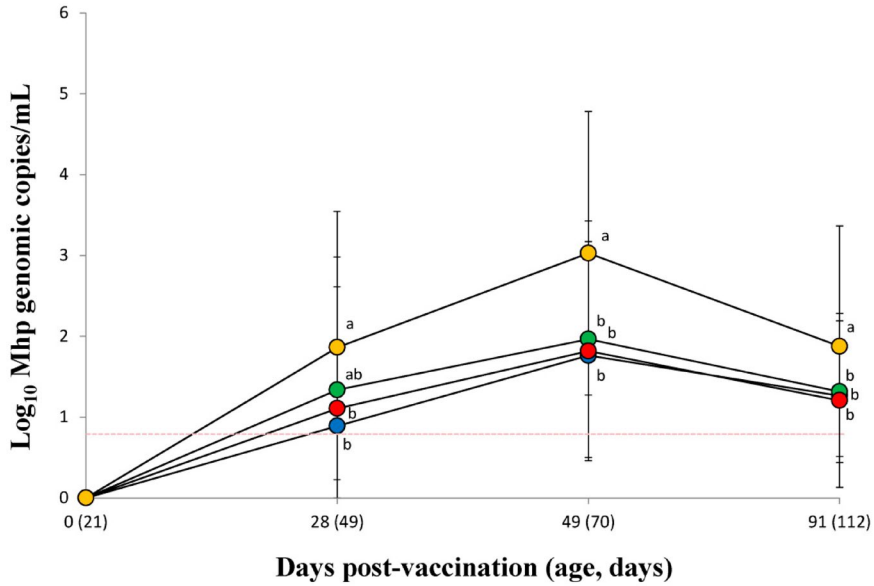
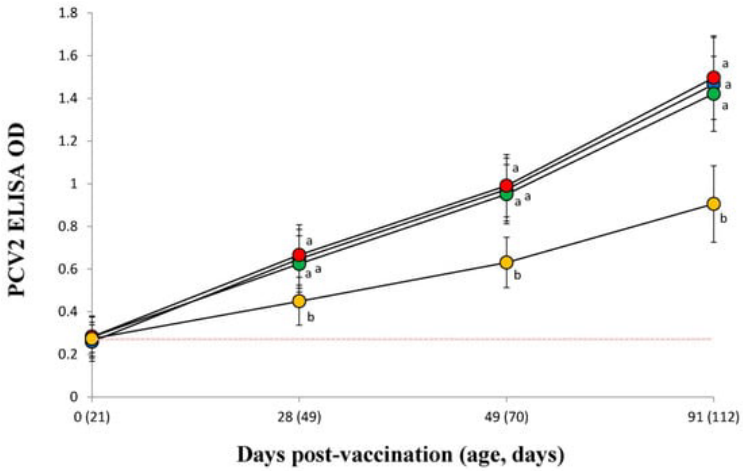
The applied vaccine was a trivalent vaccine containing porcine circovirus types 2a/b (PCV2a/b) and *Mycoplasma hyopneumoniae* (Fostera Gold PCV MH, Zoetis / CircoMax Myco) given at a dose of 2 ml. For the experiment, 240 pigs of 3 weeks of age were randomly allocated to four treatment groups of 60 pigs. In the two experimental groups, pigs were given a dose of 2 ml vaccine via needle-free devices (*VacPulse* group with Pulse FX device and in the *VacEPIG* group with the EPIG device), whereas the same dose was applied IM via needle injection (10 mm 22G) in the *VacS* group, and 2 ml saline was applied IM to pigs of the *UnVac* group.

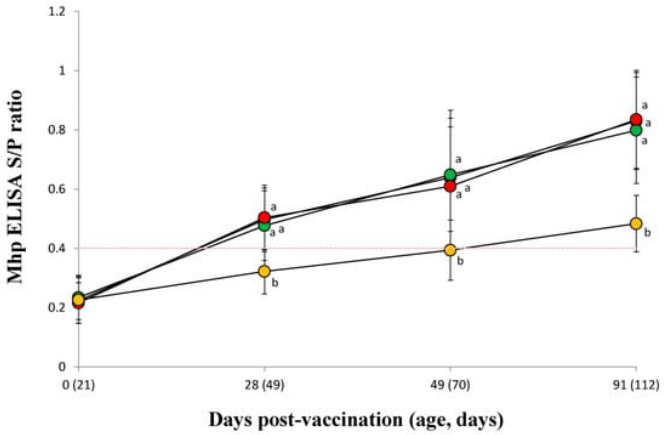
Pigs were housed in pens of 20 pigs where 5 pigs per treatment co-mingled. Observers were blinded with regard to clinical observations and pathological examinations.

Results

Parameter	***Result***
Post-vaccination skin reaction	Substantial amounts of visible vaccine residue remained on the skin at the site of vaccination for both needle-free injection devices; 30%, or 18/60 pigs from the *VacPulse* group and 20%, or 12/60 pigs from the *VacEPIG* group. No visible vaccine residues were seen in pigs from the *VacS* group. The injection site reactions were minimal for all vaccine administration types, and injection site reactions that were identified were small swellings, ranging 2–3 cm in diameter, and these self-resolved within 7 days after vaccination. Needle-free devices produced small scars in the injection site area in 4 out of 60 pigs from the *VacPulse* group and 5 out of 60 pigs from the *VacEPIG* group.
Clinical observations Pigs were monitored daily for abnormal clinical signs and scored weekly using scores ranging from 0 (normal) to 6 (severe dyspnea and abdominal breathing)	Respiratory sign scores were significantly lower ($p < 0.05$) in the vaccinated pigs (*VacS*, *VacPulse*, and *VacEPIG* groups) than those in unvaccinated pigs (*UnVac* group) at 28 to 98 days post vaccination (dpv). Respiratory sign scores of needle-free injection device-vaccinated pigs in the *VacPulse* and *VacEPIG* groups were not statistically different from those of the needle-syringe injection-vaccinated pigs within the *VacS* group.
Mortality	One, one, two and five pigs died in the *VacS*, *VacPulse*, *VacEPIG* and *UnVac* groups, respectively. The cause of death was bronchopneumonia in all cases. The microbiological examination showed mixed infection including *Pasteurella multocida*, *Glaesserella parasuis*, *Mycoplasma hyopneumoniae*, *Trueperellal pyogenes*, and PCV2d.

Average daily weight gain BW was determined at the ages of 21, 70, and 175 days, i.e. 0, 49 and 154 days after vaccination	For growth between 21 and 175 days of age as well as between 70 to 175 days of age, the ADWG of vaccinated pigs in the three vaccinated groups (*VacS*, *VacPulse*, and *VacEPIG*) was significantly higher ($p < 0.05$) than that of unvaccinated pigs (*UnVac* group). The mean AWGs of the two groups vaccinated with needle-free devices were non-inferior to conventional needle-syringe.
Quantification of PCV2d DNA in serum DNA was extracted from serum samples using a commercial kit to quantify PCV2d genomic DNA copy numbers by real-time PCR	*All three vaccinated groups showed a reduction in serum PCV2d DNA compared to the negative control group:* The amount of PCV2d DNA measured in the serum from needle-free device-vaccinated pigs (VacPulse and VacEPIG groups) was significantly ($p < 0.05$) lower at 28 dpv than that of unvaccinated pigs in the *UnVac* group. The amount of PCV2d DNA in serum of vaccinated pigs from the *VacS*, *VacPulse*, and *VacEPIG* groups was significantly ($p < 0.05$) lower at 49 and 91 dpv than that measured for unvaccinated pigs in the UnVac group. *Non-inferiority to needle injection:* Non-inferiority of the needle-free application in the *VacPulse* group to the needle injected group (*VacS*) could be demonstrated at day 49 post vaccination but not at day 91 p.v.  95%CI of VacPulse (–0.44 , 0.20) (–0.41 , 0.46) 95%CI of VacEPIG (–0.19 , 0.45) (–0.27 , 0.61) Non-inferiority Margin 0.29 0.24 **Figure 3.** The upper bound of the 95% confidence intervals (CI) on the difference in PCV2d load in serum in the needle-free device (VacPulse, •) group at 49 days post-vaccination (dpv) showed non-inferiority to conventional needle-syringe (VacS, •) group. The PCV2d loads in serum of needle-free device (VacEPIC, •) group at 49 and 91 dpv did not show non-inferiority to conventional needle-syringe (VacS, •) group.
Quantification of *Mycoplasma hyopneumoniae* DNA in larynx DNA was extracted from laryngeal swabs using a commercial kit to quantify *M. hyopneumoniae* DNA	The *M. hyopneumoniae* DNA load measured in the larynx of needle-syringe-vaccinated pigs in the *VacS* group was significantly ($p < 0.05$) lower at 28 dpv than that measured for unvaccinated pigs from the *UnVac* group. The *M. hyopneumoniae* DNA loads in the larynx of vaccinated pigs from the *VacS*, *VacPulse*, and *VacEPIG* groups were significantly ($p < 0.05$) lower at 49 and 91 dpv than that of unvaccinated pigs in the UnVac group (Figure 4).

copy numbers by real-time PCR	 **Figure 4.** Mean values of the genomic copy number of *Mycoplasma hyopneumoniae* DNA in larynx from VacS (●), VacPulse (●), VacEPIG (●), and UnVac (●) groups. Variation is expressed as the standard deviation. The detection limit of the assay is 6.3 genomic copy numbers of *M. hyopneumoniae* (red dotted line). Different superscripts (a and b) indicate significant ($p < 0.05$) different among 4 groups. *Non-inferiority:* The *M. hyopneumoniae* DNA loads in the larynx of two needle-free injection devices at 49 dpv and 91 dpv did not show non-inferiority to conventional needle-syringe injection.
Serology PCV2d Serum samples were tested by ELISA using a commercial kit. Values are OD	PCV2 antibody titres of vaccinated pigs in the VacS, VacPulse, and VacEPIG groups were significantly ($p < 0.05$) higher at 28, 49 and 91 dpv than those measured for unvaccinated pigs in the UnVac group (Figure 5).  **Figure 5.** Mean values of the PCV2 antibodies by enzyme-linked immunosorbent assay (ELISA) in serum from VacS (●), VacPulse (●), VacEPIG (●), and UnVac (●) groups. Variation is expressed as the standard deviation. Serum samples are considered positive for PCV2 antibodies if the optical density (OD) is greater than 0.3 (red dotted line). Different superscripts (a and b) indicate significant ($p < 0.05$) different among 4 groups.

	Non-inferiority: The lower bound of the 95%CI on the difference in PCV2 ELISA at 49 dpv (95%CI for VacPulse −0.07 to 0.03 and for VacEPIG −0.09 to 0.01) did not exceed non-inferiority margin (−0.10), thus demonstrating non-inferiority. The PCV2 antibody titres in the VacPulse group at 91 dpv showed non-inferiority to conventional needle-syringe injection (VacS) group (95%CI −0.10 to 0.04, non-inferiority margin −0.15).
Serology *M. hyopneumoniae* Serum samples were tested in a commercial ELISA kit. Values are expressed as sample-to-positive (S/P) ratio	*M. hyopneumoniae* antibody titres of vaccinated pigs in the VacS, VacPulse and VacEPIG groups were significantly ($p < 0.05$) higher at 28, 49 and 91 dpv than those measured for unvaccinated pigs in the UnVac group (**Figure 7**).  **Figure 7.** Mean values of the *Mycoplasma hyopneumoniae* antibodies by enzyme-linked immunosorbent assay (ELISA) in serum from VacS (●), VacPulse (●), VacEPIG (●), and UnVac (●) groups. Variation is expressed as the standard deviation. Serum samples are considered positive for *M. hyopneumoniae* antibodies if the sample-to-positive (S/P) ratio was ≥0.4 (red dotted line). Different superscripts (a and b) indicate significant ($p < 0.05$) different among 4 groups. *Non-inferiority:* The lower bound of the 95%CI on the difference in *M. hyopneumoniae* antibody titres at 49 dpv (95%CI for VacPulse −0.05 to 0.10 and for VacEPIG −0.04 to 0.11) did not exceed non-inferiority margin (−0.06). This represents the outcome that shown non-inferiority, but not superiority. The *M. hyopneumoniae* antibody titres in the VacPulse group at 91 dpv showed non-inferiority against the VacS group (95%CI −0.07 to 0.06, non-inferiority margin −0.08)
Pathology Macroscopic lung lesions were assessed by lung lobe and summed. Maximum score was 100. The severity of peribronchiolar lymphoid tissue hyperplasia by mycoplasma pneumonia	The pigs from the vaccinated group (*VacPulse*, *VacEPIG* and *VacS*) had significantly ($p < 0.05$) lower macroscopic and microscopic lung lesion scores than from pigs in the *UnVac* group (Table 3). The pigs in the vaccinated groups (*VacPulse*, *VacEPIG*, and *VacS*) had significantly ($p < 0.05$) lower microscopic lymphoid lesion scores than pigs in the *UnVac* group (Table 3). **Table 3.** Macroscopic and microscopic pathology (mean ± standard deviation) of vaccinated and unvaccinated groups.

lesions was score on a scale from 0 to 6. Lymphoid lesion severity was scored (0 to 5) based on lymphoid depletion and granulomatous inflammation	<table><tr><th></th><th colspan="4">Groups</th></tr><tr><th></th><th>VacS</th><th>VacPulse</th><th>VacEPIG</th><th>UnVac</th></tr><tr><td>Macroscopic lung lesions</td><td>16.92 ± 9.72 [a]</td><td>18.08 ± 10.34 [a]</td><td>19.66 ± 9.87 [a]</td><td>31.62 ± 11.05 [b]</td></tr><tr><td>Microscopic lung lesions</td><td>0.73 ± 0.62 [a]</td><td>0.82 ± 0.71 [a]</td><td>0.92 ± 0.68 [a]</td><td>2.06 ± 0.59 [b]</td></tr><tr><td>Microscopic lymphoid lesions</td><td>0.56 ± 0.57 [a]</td><td>0.51 ± 0.47 [a]</td><td>0.60 ± 0.52 [a]</td><td>1.76 ± 0.63 [b]</td></tr></table>Different superscripts (a and b) indicate significant ($p < 0.05$) difference among 4 groups. For the three pathology end-points a comparison between the two needle-free groups and the needle-syringe group was performed, but odds-ratios did not differ significantly.

Discussion

These results demonstrated that the outcomes of all three application modes (*VacPulse*, *VacEPIG* and *VacS*) consistently were superior to the unvaccinated (*UnVac*) group with regard to higher body weight gain and higher antibody titers against both PCV2d and *Mycoplasma hyopneumoniae*. Likewise, all vaccinated groups showed improvements in form of reduced load of PCV2d in serum, reduced load of *Mycoplasma hyopneumoniae* in larynx, reduced clinical scores and reduced lung pathology scores.

The results of this study demonstrated that vaccination via the two needle-free devices used in the study was not statistically different to that by conventional needle-syringe injection for growth performance, immune response against PCV2 and *M. hyopneumoniae*, and reduction of PCV2 viremia. Statistical non-inferiority between the needle-syringe group (*VacS*) and the two needle-free groups (*VacPulse*, *VacEPIG*) could not be demonstrated for all study parameters. One of the reasons is that demonstration of non-inferiority may require large sample sizes when the variation within groups is high.

Supportive studies

Seven clinical studies published in conference proceedings or peer-reviewed journals were submitted with the application. These studies investigated the efficacy of needle-free administration in comparison to needle-syringe administration in pigs for a variety of vaccines.

Schagemann et al. (2020) *Field testing of a new needle-free, intramuscular injection device for pigs* (p4b-schagemann-et-al-2020) ran a non-inferiority study comparing serology and viremia of a modified live PRRS vaccine when administered intramuscularly via needle versus administration via an intramuscular needle-free device. In this study, 110 PRRS negative pigs, 3 weeks of age, were vaccinated at day 0 and screened for PRRS serology and PRRS viremia at days 6 and 31 post vaccination. There was no indication that the needle-free administration was inferior to the needle vaccination based on serology. The needle-free group had 11% more viremic animals at D6 (NB: live vaccine) and an earlier onset of the immune response, and a lesser ratio of viremic animals at D31 compared to the needle group.

Cook et al. (2003) *Pulse 200 Effective Application Methods for PRRS Vaccine in Gilts* (p4b-cook-et-al-2003) compared the serological responses of gilts vaccinated with a modified live PRRS vaccine administered using conventional needles and syringes to those receiving the same vaccine with an intramuscular needle-free

device (Pulse®/Felton). Two groups of 35 animals were vaccinated at day 0 and monitored for 8 weeks post vaccination for PRRS serology. Both groups demonstrated peak responses at day 28 post vaccination, and there was no significant difference in serologic responses to PRRS modified live vaccine at any time point.

Houser et al. (2004) *Effectiveness of transdermal, needle-free injections for reducing pork carcass defects* (p4b-houser-et-al-2004) evaluated serologic responses following vaccination of 130 pigs, 4-5 weeks of age in three groups with *Mycoplasma hyopneumoniae* bacterin and pseudorabies vaccines administered via a needle syringe or needle-free device compared to unvaccinated controls. Blood samples were collected prior to vaccination, at 11–13 days following the second *M. hyopneumoniae* vaccination and at 23–25 days after the modified live pseudorabies virus (PRV) vaccination (35–36 days after the first M. *hyopneumoniae* vaccination). There were no statistical differences in serologic responses and both injection methods produced similar serological responses that were significantly greater than for unvaccinated controls.

Rosales et al. (2006) *A Comparison of the Efficacy of Conventional and Needle-free Administration of Porcilis® APP as Assessed by ELISA Concentrations of Antibody Concentration to APX I, APX II, APX III and OMP* (p4b-rosales-et-al-2006) investigated a vaccine against *Actinobacillus pleuropneumoniae* (APP) in 50 swine (finishing pigs) comparing Needle-free delivery to needle delivery. Efficacy was assessed by measuring post vaccination serum concentrations of antibodies to the toxins Apx I, Apx II, Apx III and to OMP. While the ELISA results showed statistically significant differences between Groups 1 and 2 for 2 antigens at Time 0 and for one antigen at Week 4 (NF-group slightly lower for Apx I), there were no significant differences between Groups 1 and 2 at Week 2 and 6 for any of the 4 antigens.

Thacker et al. (2003) *Safety evaluation of a modified live pseudorabies virus vaccine administered using a needle-free, transdermal injection device* (p4c-thacker-et-al-2003) investigated the serological responses in 83 sows induced by vaccination with a modified live PRV vaccine in a herd of 1700 sows, by use of a needle-free device in the rump, needle-free device in the neck and needle injection in the neck. The needle-free device performed similar to traditional injection by needle with regard to serological responses to vaccination. The needle-free-rump group had a significantly higher (i.e., better) S/P ratio (serum to positive control by ELISA) post-vaccination, but this group's S/P ratio was also higher prior to vaccination.

Gergen et al. (2002) *Intramuscular and Intradermal Vaccination of Swine for Swine Influenza Virus and Mycoplasma hyopneumoniae Using a Needle-Free Device* (p4b-gergen-et-al-2002) tested the effectiveness of different needle-free injectors with a *Mycoplasma hyopneumoniae* and SIV vaccine for 2 ml IM injection in pigs of 5-6 weeks of age. No difference in antibody titres was seen between groups administered 2 ml via needle vs. via Needle-Free device. Antibody titres were lower with 1 ml Needle-Free application than with 2 ml Needle-Free or 2 ml needle application.

Jolie and Hoover (2004) *Short-term Efficacy Study with RespiSure-One® Administered with a Needle-free Device* (p4b-jolie-and-hoover-2004) demonstrated that the administration of an inactivated *Mycoplasma hyopneumoniae* vaccine, with a needle-free injector in 3-week-old pigs was efficacious against an experimental *M. hyopneumoniae* challenge. Thus, lung lesion scores were reduced by 88.4% compared to the negative control group, and 100% of the vaccinated group tested positive at days 56 post vaccination compared to 45% in the negative control group. Safety was demonstrated by the absence of any apparent tissue damage at the time of necropsy and significantly fewer lung lesions in the vaccinated than the placebo pigs. The control group of this study was administered saline by needle injection.

Overall, the seven attached clinical studies from different publications compare needle-free administration of a number of pig vaccines with traditional syringe-needle application. In general, these studies demonstrate that the efficacy of application via needle-free devices similar to the efficacy after traditional needle-syringe application. The pigs in the different experiments were 3 weeks of age or older upon vaccination; therefore, the recommendation given in the product information should be that needle-free administration can be used for pigs at 3 weeks of age or older.

3. Scientific Overview

In support of this variation, the applicant has provided three proprietary studies aimed to demonstrate the capability of a needle-free device to apply the desired dose of vaccine intramuscularly. The company has furthermore provided a pivotal field study from an independent peer-reviewed publication to demonstrate non-inferiority of application of CircoMax Myco by two devices compared to traditional needle-syringe injection. Finally, in support of the general suitability of needle-free application for different pig vaccines, the applicant has carried out a literature review and provided 7 publications.

The MAH has proposed to include the following text in the SPC section 3.9: 'The use of a multi-dosing syringe or a needle-free device for intramuscular injections is recommended. In each case, use vaccination devices according to the manufacturer's instructions. For needle-free administration, use a needle-free device appropriate to deliver intramuscular injections of 1 and/or 2 ml doses in the relevant ages of pigs. Follow manufacturer's instructions specific to the pressure required to administer the required dose volume, and specific to handling and cleaning processes. Follow any restriction imposed by the device manufacturer specific to animal age or body weight limits.'

Pre-clinical trials

Three studies on tissue distribution of dye-marked injectables after injection via syringe-needle or via the needle-free device were carried out:

> Evaluation of the Deposition of 0.5 mL Methylene Blue Dye Administered in the Neck Muscle of Neonatal Pigs via a Needle-Free Injection Device (B920R-US-22-B80)
>
> Evaluation of the Deposition of 1.0 mL Methylene Blue Dye Administered in the Neck Muscle of 21 Day of Age Pigs via a Needle-Free Injection Device (B920R-US-22-B81)
>
> Evaluation of the Deposition of 2.0 mL Methylene Blue Dye Administered in the Neck Muscle of 21 Day of Age Pigs via a Needle-Free Injection Device (B920R-US-22-B82)

In these three studies, a mixture of methylene blue in PBS and 10% SP oil was applied to the pigs via either traditional needle-syringe application or a need-free device (Pulse® FX). The mixture was chosen to mimic the viscosity of different swine vaccine from the applicant. A question has been raised for the applicant to provide a detailed comparison of the physical characteristics of the oil-dye-PBS mixture and the CircoMax Myco vaccine, and to specify the content of the "SP oil".

For each study at least 30 pigs were included. Both delivery methods were used on each pig and randomly allocated to different sides of the neck. The pigs were euthanised immediately after injection and a necropsy was performed on the injection sites. The tissue distribution of the dye solution was measured with regard to injection depth and percent distribution of dye into the dermis, subcutis, and muscle was recorded.

In all three studies, 100% of the dye was present in the muscle when the oil-dye mixture was given via the traditional needle-syringe method. The percent present in the muscle after needle-free application was 99.96, 98.1, and 95.6 % in the studies applying 0.5 ml at 1-3 days of age, 1 ml at 3 weeks of age and 2 ml at 3 weeks of age, respectively. The studies seem to demonstrate the relative tissue distribution of the dye which had actually entered the animal. The applicant concluded that the amount of dye present on the skin surface was negligible.

In conclusion, it was demonstrated that the needle-free device was able to deliver a consistent proportion of the dose into the muscle tissue in pigs of 3 weeks of age or older.

Field trial

Cho H, Ahn Y, Oh T, Suh J, Chae C. Non-Inferiority Field Study Comparing the Administrations by Conventional Needle-Syringe and Needle-Free Injectors of a Trivalent Vaccine Containing Porcine Circovirus Types 2a/2b and *Mycoplasma hyopneumoniae*. Vaccines (Basel). 2022 Feb 24;10(3):358. doi: 10.3390/vaccines10030358. PMID: 35334988; PMCID: PMC8952852.

A clinical study was carried out by Cho et al. (2002) with the aim to demonstrate non-inferiority between application methods of Fostera® Gold PCV MH, Zoetis (a trade name for CircoMax Myco) for a range of efficacy parameters. Reactions at the injection site were also compared. The study was carried out on a commercial farm, pigs were randomised to treatment group, and clinical and pathological readings were blinded. GCP status was not stated.

Sixty 3-week-old animals per treatment group were vaccinated IM with a 2 ml dose of the PCV2a/PCV2b/M. hyo via Pulse® FX needle-free device (*VacPulse* group) or EPIG® needle-free device (*VacEPIG*), or via conventional needle and syringe (*VacS*). Control group animals were administered a 2 ml dose of PBS using a needle and syringe device (*UnVac* group). The pigs were reared to the age of 175 days. Pigs were observed for clinical scoring (weekly), body weight was determined at 21, 70, and 175 days of age, laryngeal swaps for *Mycoplasma* PCR were taken at 21, 49, 70 and 112 days of age and serum samples were taken for ELISA and PCV2d PCR at same time points.

Injection site reactions in the pigs were minimal for the two needle-free injection devices and needle-syringe injection consisting of swellings 2-3 cm in diameter which resolved within 7 days after vaccination. Small scars were detected at the injection site in 4 out of 60 pigs from the *VacPulse* group and 5 out of 60 pigs from the *VacEPIG* group.

The growth performance of the two groups where pigs were vaccinated with the needle-free devices was non-inferior to pigs vaccinated via needle-syringe application.

Respiratory sign scores of needle-free injection device-vaccinated pigs in the *VacPulse* and *VacEPIG* groups were not statistically different from those of the needle-syringe vaccinated pigs in the *VacS* group, and all three vaccinated group had significantly lower scores than the unvaccinated group. One, one, two and five pigs died in the *VacS*, *VacPulse*, *VacEPIG* and *UnVac* groups, respectively. The cause of death was bronchopneumonia.

Vaccination of pigs with the two needle-free injection devices reduced levels of PCV2d loads in serum and *M. hyopneumoniae* loads in the larynx equally compared to the conventional needle-syringe injection. No statistical difference was detected between the vaccinated groups and non-inferiority was confirmed for PCV2d load in serum from the needle-free Pulse FX injection device at 49 days post vaccination.

The immune response against PCV2 and *M. hyopneumoniae* to vaccination via the needle-free Pulse FX injection device was non-inferior to conventional needle-syringe injection.

The pigs from the two needle-free injection device and conventional needle-syringe injection had significantly ($p < 0.05$) lower macroscopic and microscopic lung lesion scores at necropsy, and microscopic lymphoid lesions than unvaccinated pigs, and no significant difference was detected between the vaccinated groups.

The results of this study demonstrated that vaccination via the two needle-free devices used in the study was non-inferior to that by conventional needle-syringe injection with regard to growth performance, reduction of PCV2d viremia, PCV2d serology and *M. hyopneumoniae* serology. Additionally, respiratory sign scores as well as macroscopic and microscopic lung lesion scores showed no statistical difference between the vaccination devices, although statistical non-inferiority was not demonstrated.

Supportive studies

The applicant presented a total of 16 supportive studies in relation to vaccination via needle-free devices. These pre-clinical and clinical studies published in conference proceedings or peer-reviewed journals investigated the efficacy of needle-free administration in comparison to needle-syringe administration in pigs for a variety of vaccines.

As these papers generally describe the results from vaccines other than CircoMax Myco and CircoMax, they will not be described in detail here.

In general, the studies showed very similar efficacy results between application of the vaccines via needle-free devices compared to conventional needle-syringe application, and do not give reason for safety concerns in pigs of the age of 3 weeks or above. None of these studies, however, investigated the use in the youngest category of pigs to be vaccinated with CircoMax and CircoMax Myco, i.e. the pigs of 3 days of age vaccinated under the "split-dose" schedule.

The papers also supported the background for using needle-free devices. The method may have animal welfare advantage over needle-syringe vaccination due to a lower pain response. The method may also lead to a lower incidence of injuries at the injection site, which may be beneficial both from the animal welfare perspective and in relation to carcass quality. Furthermore, there was evidence from one study that the needle-free application could reduce but not eliminate the risk of haematogenous pig-to-pig transmission of PRRSV.

Overall, the data presented are supportive of needle-free administration of CircoMax and CircoMax Myco to pigs of 3 weeks of age or older.

4. Benefit-risk assessment of the proposed change

CircoMax is authorised for the active immunisation of pigs against porcine circovirus type 2 to reduce viral load in blood and lymphoid tissues, faecal shedding and the lesions in lymphoid tissues associated with PCV2 infection (porcine circovirus types 2a, 2b and 2d). The onset of immunity is 3 weeks and the duration of the immunity is 23 weeks.

CircoMax Myco is authorised for the active immunisation of pigs against porcine circovirus type 2 to reduce viral load in blood and lymphoid tissues, faecal shedding and the lesions in lymphoid tissues associated with PCV2 infection (porcine circovirus types 2a, 2b and 2d and against *Mycoplasma hyopneumoniae* to reduce the lung lesions associated with *Mycoplasma hyopneumoniae* infection. The onset of immunity is 3 weeks and the duration of the immunity is 23 weeks. In addition, vaccination has been shown to reduce body weight gain losses under field conditions.

The proposed variation is to add the option of administering CircoMax Myco and CircoMax intramuscularly, using needle-free devices. The applicant has proposed to amend the SPC section 3.9 with the following text:

'The use of a multi-dosing syringe or a needle-free device for intramuscular injections is recommended. In each case, use vaccination devices according to the manufacturer's instructions. For needle-free administration, use a needle-free device appropriate to deliver intramuscular injections of 1 and/or 2 ml doses in the relevant ages of pigs. Follow manufacturer's instructions specific to the pressure required to administer the required dose volume, and specific to handling and cleaning processes. Follow any restriction imposed by the device manufacturer specific to animal age or body weight limits.'

Additionally, some editorial changes to the SPC/PI are proposed.

4.1. Benefit assessment

Direct therapeutic benefit

The benefits of the product remain unaffected by this variation.

Additional benefits

Additional benefits of needle-free application over conventional needle-syringe application have not been investigated extensively, but may include:

- Animal welfare may benefit due to a lower level of pain inflicted by the needle-free application.
- Needle-free application of vaccines may lead to a lower risk of transmitting pathogens between pigs, although the risk will not be completely eliminated.
- Less risk of needle-stick injuries to the user (provided that the needle-free device is used correctly)
- Less risk of needle injuries to the pig, which will be of benefit to animal welfare and carcass quality.

4.2. Risk assessment

Quality:

Quality remains unaffected by this variation.

Safety:

Risks for the target animal:

Administration of CircoMax and CircoMax Myco in accordance with SPC recommendations for needle-free application was generally well tolerated in pigs above 3 weeks of age in the bibliographic study (Cho et al. 2022) and in studies applying dye solutions.

Otherwise, the risk for the target animal will not be affected by this variation or may be reduced by the needle-free application due to lower risk of injection site damage. The needle-free application may also have welfare advantages over needle-syringe application.

Risk for the user:

It is not entirely certain whether needle-free application will reduce the risk for the personnel responsible for applying the vaccine, but most likely the risk of self-injection will be reduced. This risk will be related to the specific device to be used.

Risk for the environment:

No changes in the risk for the environment are expected.

Risk for the consumer:

Needle-free application will eliminate the risk of fragmented needles in the injection site. Otherwise, the risk for the consumer will not be affected.

4.3. Risk management or mitigation measures

Vaccination via needle-free devices has been demonstrated to be safe for pigs of 3 weeks of age or older. Section 3.9 of the SPC has been updated to state "The use of a multi-dosing syringe or a needle-free device for intramuscular injections is recommended. In each case, use vaccination devices according to the manufacturer's instructions. For needle-free administration use a needle-free device appropriate to deliver intramuscular injections of 2 ml dose in pigs from 3 weeks of age. Follow manufacturer's instructions specific to the pressure required to administer the required dose volume, and specific to handling and cleaning processes. Follow any restriction imposed by the device manufacturer specific to animal age or body weight limits".

4.4. Evaluation of the benefit-risk balance

Based on the data presented, the overall benefit-risk balance of the variation is considered positive.

5. Conclusion

Based on the original data presented on safety and efficacy, the Committee for Veterinary Medicinal Products (CVMP) concluded that the application for variation to the terms of the marketing authorisation for CircoMax and CircoMax Myco is approvable.

Changes are required in the following Annexes to the Community marketing authorisation.

I, IIIA and IIIB

Please refer to the separate product information showing the tracked changes.

As a consequence of this variation, sections 3.6, 3.9, 5.3 and 6 of the SPC are updated. The corresponding sections of the Package Leaflet are updated accordingly.