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# The potential role for BCG vaccination in global efforts to control and eradicate bovine tuberculosis

A technical discussion paper compiled by the OIE Reference Laboratory for Bovine Tuberculosis based at the United Kingdom's Animal and Plant Health Agency (Weybridge)

## Introduction

Bovine tuberculosis is a chronic animal disease caused by infection with members of the *Mycobacterium tuberculosis* complex, primarily *M. bovis*. It is also a major zoonotic disease. The World Health Organisation (WHO) has set a goal of eradicating human TB by 2030. The WHO, World Organisation for Animal Heath (OIE) and the Food and Agriculture Organisation (FAO) have published a Zoonotic TB Roadmap to support this goal (WHO and others, 2017a)

The UK Animal and Plant Health Agency (APHA) is an OIE Reference Laboratory for Bovine Tuberculosis jointly supported by the UK Government's Department of Food and Rural Affairs (Defra) and the devolved administrations in Scotland and Wales. APHA is working towards a deployable cattle TB vaccine and associated DIVA test to detect infected among vaccinated animals as part of efforts to eradicate bovine TB in the UK.

National control and eradication programs based on test and slaughter of infected animals have been successfully implemented in many countries. However, this approach can have limitations and the UK believes that a cattle TB vaccine has the potential to be a valuable additional tool in global efforts to control and eradicate this disease. This paper outlines the strategy, underlying science, research and regulatory steps that are being undertaken in the UK to reach the goal of a deployable, commercial, veterinary-authorised BCG (Bacille Calmette-Guérin) vaccine for cattle and companion DIVA test.

The path to a deployable cattle TB vaccine is underpinned by over two decades of research and development work carried out at APHA in collaboration with, and alongside, other research teams from around the world.

The proposed bovine TB vaccination strategy is based on:

- Vaccination by subcutaneous injection of BCG (BCG Danish SSI 1331).
- Annual revaccination.
- The complementary use of an associated DIVA (Detection of Infected amongst Vaccinated Animals) Skin Test (DST) to identify infected animals within a vaccinated population (vaccinated herds) instead of the conventional tuberculin skin test.

## What is the UK's aspiration?

The UK's aspiration is for international recognition of these additional tools to support the global eradication of bovine TB and APHA stands ready to support the OIE in this endeavour. APHA warmly welcome any feedback or discussion.

# Bovine TB

Bovine tuberculosis is a chronic bacterial disease of animals caused by members of the *Mycobacterium tuberculosis* complex, primarily by *M. bovis*. It is a major zoonotic disease, and cattle are the main source of infection for humans. The WHO has set a goal of eradicating human TB by 2030. *M. bovis* is estimated to account for up to 10% of human TB cases in some low- and medium-income countries (LMIC) mainly through consumption of unpasteurised milk or milk products. In 2016, there were an estimated 147,000 new cases of zoonotic TB in people globally, and 12,500 deaths due to the disease (WHO and others, 2017b). However, the true burden of zoonotic TB is likely to be underestimated.

Bovine tuberculosis is found throughout the world, but some countries have never detected the disease and many developed countries have reduced or eliminated it from their cattle population. Other countries have substantially reduced the prevalence and confined it to a few infected zones. However, significant pockets of infection remain in wildlife in some countries. The highest prevalence of bovine tuberculosis is in Africa and parts of Asia, but the disease is also found in countries in Europe and the Americas.

National control and eradication programs based on traditional test and slaughter of infected animals have been successfully implemented in many countries. However, this approach remains impractical in some heavily infected countries, because it necessitates slaughtering large numbers of cattle. This may not be feasible, due to human resource or financial limitations within the national animal health programmes, or for cultural, social or religious reasons. The OIE has recently established an ad hoc Group on alternative strategies for the control and elimination of Mycobacterium tuberculosis complex infection in livestock. The objective of this Group is to explore and recommend actionable strategies to control tuberculosis in livestock in areas where the slaughter of cattle as disease control measure is not an option, and the tuberculosis burden in humans is still unacceptably high.

Vaccination of cattle with BCG, supported by a test that can accurately detect infected animals among the vaccinated population, offers an exciting new approach to controlling and eradicating this complex and challenging disease.

# BCG

## Introduction

BCG is a live, attenuated strain of *Mycobacterium bovis* isolated by Emile Nocard from a cow and attenuated by Calmette and Guerin over 200 passages from the virulent strain performed over eight years, until it was observed to be no longer capable of causing disease in guinea pigs, horses, and cattle (Calmette & Guérin 1913). Molecular genetic analyses reveal that BCG underwent an irreversible deletion of a significant 9.5 Kb region (RD-1) containing nine key virulence genes (Behr et al. 1999). It is one of the most widely

used human vaccines globally and has an unparalleled safety record, having been used in targeted human populations for over 100 years with up to 100 million doses annually. Despite the large number of doses (5 billion) delivered since its first usage in 1921, reports of adverse reactions arising from the use of the BCG in humans are rare. Importantly, it is considered safe across all age groups except immune-compromised individuals. Neonatal BCG vaccination is recommended by the World Health Organisation (WHO) for all children living in TB endemic areas, apart from those immunocompromised by human immunodeficiency virus (HIV) (WHO, 2008). A review of the history and impact of BCG has recently been published (Ahmed et al. 2021)

**BCG use in cattle vaccination**

The use of BCG for vaccination of cattle against bovine tuberculosis also has a long history, with Calmette & Guérin reporting studies as early as 1911. These and other historical studies (summarised by Buddle et al, 2018) indicated that BCG could induce protection against TB although this was not absolute, and it appeared to wane after 1-2 years. Furthermore, vaccination of cattle with BCG could induce positive reactivity to tuberculin skin testing. Progress towards the development of a DIVA test that could detect infected amongst vaccinated animals (see later) has reinvigorated interest in the use of BCG as a vaccine against BTB. In its 2020 response to the recent independent review of the TB eradication strategy for England (Godfray et al. 2018) the UK Government committed to acceleration of work to develop a deployable cattle TB vaccine. The UK Government considers that deployment of a cattle bovine tuberculosis vaccine would complement current measures such as cattle testing, movement restrictions on infected herds and the rapid detection and removal of infected animals (Defra, 2020). But it is important to note that no single measure will provide a long-term solution.

**Many studies in the past 25 years have confirmed BCG can induce effective protection against TB**

More recently, a sizable number of studies in the past 25 years using vaccination/challenge experiments (where vaccinated or control animals are experimentally infected with *M. bovis*) provide significant evidence that BCG can induce effective protection of cattle against TB (reviewed by Buddle et al., 2018). These studies reveal that BCG vaccination offers a spectrum of protection against BTB. 25 to 30% of vaccinated animals are ‘fully protected’ (absence of visible TB lesions and/or TB culture); around 30% display partial protection (reduced severity of TB pathology when compared to non-vaccinated controls), and the remainder are unprotected.

For example, data from six efficacy studies performed at APHA (summarised in Figure 1) illustrate an overall reduction in pathology score of ~70% seen in vaccinated animals compared to controls based on a semi-quantitative pathology scoring system (Vordermeier et al. 2002).

Reduction of overall pathology may also play an indirect role in vaccine efficacy by reducing the potential for transmission by vaccinates after infection. Full protection overall

in this set of experiments amounted to about 30 %, which is in the same order of magnitude as published recently in a systemic review of BCG efficacy (Srinivasan 2021)

It is important to note however, that these outcomes are seen in an experimental model, where all animals are challenged with a much larger dose than would be feasible under natural infection pressure. Further, such models do not allow appreciation on the degree onward transmission would be reduced by BCG vaccination. The need to statistically power such studies requires that all control (non-vaccinates) animals develop pathology, resulting in such models being highly stringent, with very low possibility of achieving sterilising immunity.

These considerations are not only relevant to direct infection studies using, for example, endobronchial instillation of *M. bovis* but also to experiments of natural transmission in-contact infection in cattle as carried out in Ethiopia (Ameni et al. 2010 and 2018; summarised in Table 1). Interestingly, in these studies, showed that BCG vaccination led to a statistically significant reduction of overall pathology combined with a proportion of calves presenting without signs of pathology ('fully protected'). Thus, they are broadly in line with the studies using direct challenge summarised in Figure 1.

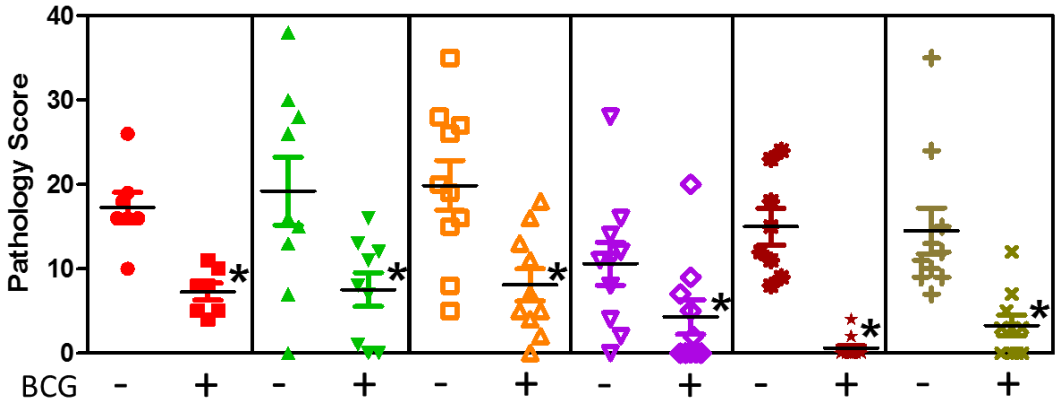
**Field trials of BCG vaccination have demonstrated efficacy in reduction the number of cattle infected with *M. bovis* in natural transmission settings**

Recently, BCG vaccination field trials have been conducted in cattle in Mexico, New Zealand and Chile (Table 2) where the effect of vaccination (three subcutaneous delivery vaccine trials and one oral delivery vaccine trial) has been assessed under natural transmission pressure. Field trials, in contrast to experimental infection studies described in the previous section, can not only show the direct effect of vaccination (i.e. the reduction of the proportion of susceptible animals) but will also take secondary effect (reduction of onward transmission) into account. These trials demonstrate that BCG vaccination can markedly reduce the number of cattle which become infected, and subsequently develop disease. The outcomes of these trials are different to that seen in vaccination/experimental challenge studies where vaccination primarily reduces the severity of the disease. These studies are important as they provide critical evidence of the likely impact of BCG vaccination strategies in "real world" situations.

**Modelling the impact of BCG vaccination on TB prevalence**

To assess the potential impact of BCG vaccination of cattle on herd level prevalence of bovine tuberculosis, a recent systematic review of published BCG efficacy studies and trials performed scenario analyses (intensification of production in LMIC) using transmission dynamic models incorporating direct and indirect vaccine effects (Srinivasan et al., 2021). This analysis suggested that the disease in low to moderate (<15%) prevalence settings could be reduced close to "TB-Free" (free from infection) levels if cattle BCG vaccination alone (i.e. not combined with other control strategies) were introduced in the next 10-years. Furthermore, immediate implementation of BCG vaccination may result

in 50–95% of cumulative cases being averted over the next 50 years even in high (20–40%) disease burden settings.




| Experiment | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Age (weeks) | < 4 | < 4 | 35 - 43 | 35 - 43 | 22 - 30 | 17-26 |
| Interval (months) | 3 | 12 | 3 | 3 | 5-7 | 3 |

**Fig. 1 Summary of BCG vaccination/challenge studies conducted at APHA**

*Indicates statistically significant protection $p<0.05$ between BCG vaccinated and control groups (Mann-Whitney test)

**Table 1 Summary of two natural transmission in-contact infection studies carried out in Ethiopia**

| Vaccine route | BCG Strain | Vaccine Dose | Source of infection | Age at vaccination | Measurement of disease | Assessment<br><br>BCG vs. Control | Reference |
|---|---|---|---|---|---|---|---|
| S/C | Danish | $10^6$ | Infected herd | 2 weeks | Proportion with TB lesions<br><br>Mean total pathology score (95% CI) | 5/13 vs.12/14<br>38% vs. 86%<br><br>4.6 (0-10.5) vs 14.1 (2.5-24.6) | Ameni et al. (2010) |
| S/C | Danish | $10^6$ | Infected herd | 2 weeks | Proportion with TB lesions<br><br>Mean total pathology score (95% CI) | 15/23 vs.22/26<br>65% vs. 85%<br><br>4.0 (1.8-6.1) vs 7.8 (2.5-13.1) | Ameni et al. (2018) |

S/C, Subcutaneous, CI confidence interval

**Table 2 Summary of field trials which assessed the efficacy of BCG vaccine against bovine tuberculosis**

| Country | BCG Strain | Vaccine Dose | Vaccine route | Source of infection | Age at vaccination | Measurement of disease | Assessment<br><br>BCG vs. Control | Reference |
|---|---|---|---|---|---|---|---|---|
| Mexico | Tokyo | $10^6$ | S/C | Infected herd | 1-2 weeks | Proportion positive in three tests; PPD skin test, PPD and ESAT-6 /CFP-10 IGRA | 6/64 vs 15/66<br><br>9.4% vs 22.7%<br><br>59.4% efficacy | Lopez-Garcia et al. (2009) |
| New Zealand | Danish | $10^8$ | Oral | Infected herd and wildlife | 1-4 years | Proportion with TB lesions and/or *M.bovis* cultured | 31/644 vs. 63/531<br><br>4.8% vs. 11.9%<br><br>67.4% efficacy | Nugent et al. 2017 |
| New Zealand | Danish | $3 \times 10^5$ | S/C | Infected herd and wildlife | 1-2 years | Proportion with TB lesions and/or *M.bovis* cultured | 2/520 vs. 8/297<br><br>0.38% vs. 2.69%<br><br>85.7% efficacy | Nugent et al. (2018) |
| Chile | Russia | $2 - 8 \times 10^5$ | S/C | Infected herd and wildlife | 11 months | Proportion positive to ESAT-6 /CFP-10/Rv3615c IGRA | 7/62 vs. 17/60<br><br>11.3% vs. 28.3%<br><br>66.5% overall efficacy | Retamal et al. (2021) |

S/C, Subcutaneous; IGRA, Gamma-interferon release assay; PPD, Purified protein derivative

## Safety of BCG vaccination in cattle

APHA has conducted four studies (undertaken following Good Laboratory Practice guidelines) examining the safety of BCG vaccination of cattle (Table 3). These included safety in pregnant heifers at each trimester and in lactating cows, including analysis of shedding of BCG in milk and milk yield and calves. Factors such as the effect of a ten-fold overdose of BCG and repeat doses was examined. The overall conclusion of the studies was that BCG vaccination produced no detrimental effects; in fact, the milk yield was raised in the vaccinated, lactating animals. This additional benefit has also been confirmed in a recent field trial in Chile where significantly increased milk production in the 100 day

period post-partum was observed in BCG vaccinates compared to unvaccinated animals from the same herd (Retamal et al., 2021).

**Table 3 Summary of BCG GLP safety studies carried out in cattle at APHA**

| Study | 10 × overdose | Repeat dose | Single dose | Conclusion |
|---|---|---|---|---|
| Pregnant heifers (each trimester) | X | X | X | Safe |
| Calves 1 | X | X | X | Safe |
| Calves 2 | | | X | Safe |
| Lactating cows (including milk shedding and yields) | | | X | Safe |

APHA has also carried out a quantitative risk assessment of the safety of BCG vaccination in cattle (APHA, 2019). This included consideration of areas such as biological properties of BCG, genetic stability and potential for reversion to virulence, shedding and survival of BCG and pathogenicity to other species. The conclusion was that “the overall risk of damage to the environment by the use of BCG Danish in cattle is concluded to be effectively zero”. This risk assessment was submitted to the UK Veterinary Medicines Directorate (VMD) as part of an Animal Test Certificate application to perform BCG vaccination field trials (see below) and was approved by the UK Food Standards Agency’s Advisory Committee on the Microbiological Safety of Food (ACMSF). The VMD has requested a precautionary 90-day meat and offal withdrawal period for use of the BCG vaccine during the forthcoming UK field trials i.e. meat and offal from vaccinates cannot enter the food chain for 90 days post-administration.

## DIVA TEST

### Why is a DIVA test needed?

It is well established that vaccination with BCG can sensitise cattle to bovine tuberculin and compromise the specificity of the tuberculin skin test, which currently serves as the primary surveillance test for “test and slaughter” bovine TB control strategies worldwide

(Vordermeier et al. 2011a). As a result, DIVA tests will be required for countries intending to use BCG vaccination alongside conventional “test and slaughter” programmes. Such DIVA tests will allow identification of infected animals including those in which BCG vaccination has failed to prevent infection, while minimising the risk of false positive results induced by the vaccine (Vordermeier et al. 2011b).

**DIVA testing strategy: use antigens that result in positive tests in infected animals but not in vaccinated animals**

The basis of the DIVA test proposed by the APHA is the use of three antigens (ESAT-6, CFP-10 and Rv3615c) that induce an immune response in *M. bovis* infected animals (and therefore results in a positive skin test result) but not in BCG vaccinates – because either the gene coding for these proteins is absent from, or the protein is not secreted by, BCG (Vordermeier et al. 2016; Sidders et al. 2008). The antigens in question can be synthetically produced as recombinant proteins or peptides and mixed together as a cocktail.

Originally the DIVA test was envisaged to be based on the whole blood interferon-gamma release assay (IGRA) modified with these DIVA antigens (Vordermeier et al. 2016). However, modelling by Conlan et al. 2015 suggested that very high specificity (>99.85%) from any DIVA test would be required for vaccination to be economically viable. As this high level of specificity could not be obtained with the IGRA test, attention turned towards the use of the DIVA antigens in a skin test platform as first described by Whelan et al. (2010).

**The current proposed DIVA test is based on a synthetic fusion protein in a skin test format analogous to the current tuberculin skin test**

Initial ‘proof of concept’ data was generated using a protein cocktail consisting of the three individual recombinant proteins for ESAT-6, CFP-10 and Rv3615c. The DIVA antigens have been developed further into a single synthetic fusion protein formulation designated DST-F (DIVA Skin Test – Fusion), where the three individual DIVA proteins have been produced as one recombinant fusion protein (Srinivasan et al. 2019). This approach simplifies manufacture and quality control.

The DST-F reagent is used in a skin test format directly analogous to the use of tuberculin skin test. The DST-F skin test principle and procedure is the same, i.e. to elicit a delayed-type hypersensitivity reaction in *M. bovis*-infected animals approximately 72 hours after injection of the reagent into the skin of the neck. The only difference to the current tuberculin skin test is the formulation of the reagent (DST-F instead of Purified Protein Derivative of bovine tuberculin) and that a simultaneous injection of avian tuberculin (for comparison) is not required to achieve high specificity in the test.

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**Validation of the DST-F skin test – promising results to date**

The validation of DST-F skin test and determination of test sensitivity and specificity is currently based on testing cattle of known TB infection and vaccination status in an experimental (as opposed to field) setting and comparing the resulting DST-F skin test results.

To determine specificity (probability that a test will correctly identify an animal that is free from infection as test negative) test result data are required from BCG vaccinated cattle and unvaccinated control cattle. To determine sensitivity (probability that a test will correctly identify an infected animal as test positive), test result data are required from *M. bovis* infected cattle (naturally and experimentally infected).

A summary of the DST-F test data generated to date at APHA (Table 4) demonstrates that the DST-F skin test shows promising results and has been shown to be effective for detection of infected animals while giving negative results in uninfected cattle (including those vaccinated with BCG).

**Table 4 Summary of the performance of the DST-F skin test**

| | % Specificity [95% CI] (number positive / total number) | | % Sensitivity [95% CI] (number positive / total number) | |
|---|---|---|---|---|
| **Test** | BCG vaccinated | Unvaccinated controls | *M. bovis* Infected [a] | BCG vaccinated experimentally infected |
| **DST-F [b]** | 99 [92, 100] (1 / 70) | 100 [95, 100] (0 / 70) | 92 [85, 96]* (102 / 111) | 85 [76, 91]** (73 / 86) |
| **SICCT [c]** | 78 [66, 87] (13 / 60) | 100 [94, 100] (0 / 60) | 84 [76, 89] (93 / 111) | 81 [72, 88] (69 / 85) |
| **SIT [d]** | 38 [27, 51] (37 / 60) | 100 [94, 100] (0 / 60) | 98 [94, 100] (109 / 111) | 100 [96, 100] (85 / 85) |

[a] Consist of naturally and experimentally infected animals
[b] Response considered positive if Δ skin thickness is ≥ 2mm
[c] Response considered positive if Δ skin thickness for PPDB – PPDA is > 4mm (standard OIE and UK test interpretation)
[d] Response considered positive if Δ skin thickness for PPDB is ≥ 4mm
* $p < 0.05$, ** $p < 0.01$, McNemar's test (compared to SIT)

When compared to the current single intradermal comparative cervical test (SICCT) and single intradermal cervical tests (SICT), where all three test results were generated concurrently in the same individual animals, the DST-F shows close to perfect specificity in

BCG vaccinated uninfected animals. In contrast, and as expected, the SICT and SICCT test show some cross reaction in those same animals. No unvaccinated control animals tested positive with DST-F, matching results from the SICT and SICCT test.

In terms of sensitivity, the DST-F skin test identified 92% of *M. bovis* infected animals and 85% of animals that had been experimentally infected following BCG vaccination (as noted above, experimental infection tends to overwhelm vaccination even if the severity of the disease is reduced). These sensitivity values were better than for the SICCT test but slightly reduced compared to the SICT test.

APHA plan to expand this validation data set as new research and field studies are completed (see below), prior to submission of the DSF-F data dossier to OIE for consideration.

**Safety of DST-F in cattle**

APHA has conducted a GLP (good laboratory practice) study examining the safety of repeated administration of one dose of DST-F in both BCG vaccinated and unvaccinated calves. The observations of this study demonstrated that the intradermal administration of the DST-F, either alone or concurrently with PPD administration, in unvaccinated calves and BCG vaccinated calves did not cause any adverse local effects.

**Moving towards marketing authorisations - current and future APHA studies**

The aim of this programme of work is to secure UK marketing authorisations for BCG (CattleBCG) and the complementary DIVA test (DST-F). APHA plan to submit marketing authorisation applications for both products to the VMD.

Marketing authorisation application dossiers will be reliant on data demonstrating BCG vaccine safety and DIVA skin test safety and efficacy in a field trial situation and will follow a phased five-step process (Figure 2).

In July 2020, VMD granted APHA Animal Test Certificates (ATC) which authorise APHA to generate this field trial data (completing Step 1). This was based on submission of ATC applications to VMD which contained the following information:

- Manufacture details (as for human TB, supplied by SSI)
- Seven experimental vaccination/infection studies
- Four GLP safety studies
- Quantitative Risk assessment

Step 2 is a field trial for DST-F in unvaccinated controls to confirm safety and specificity of DST-F in a larger number (300 – 1000) of animals. Target specificity (lower 95% CI) is > 98%. This field trial began in June 2021. In addition, the sensitivity of DST-F will be

assessed in a separate study using a large number (approximately 300) of naturally infected animals (SICCT test reactors from known infected herds) transported to APHA Weybridge for follow-up DST-F testing.

A second field trial (Step 3) will focus on cattle BCG safety combined with the safety and specificity of DST-F in vaccinates. This will involve 1900 animals with an equal proportion of vaccinates and controls. This field trial is expected to start in early 2022.

At present it is estimated that application for marketing authorisation will be completed in mid-2023. In addition, APHA is performing an additional experimental study to further support a one-year duration of immunity claim for the vaccine used at the minimum dose.

There are also parallel programmes of work; to secure commercial scale GMP manufacturing of the DST-F and development of complementary analytical tools for characterisation, reference standards and quality control of this key reagent.

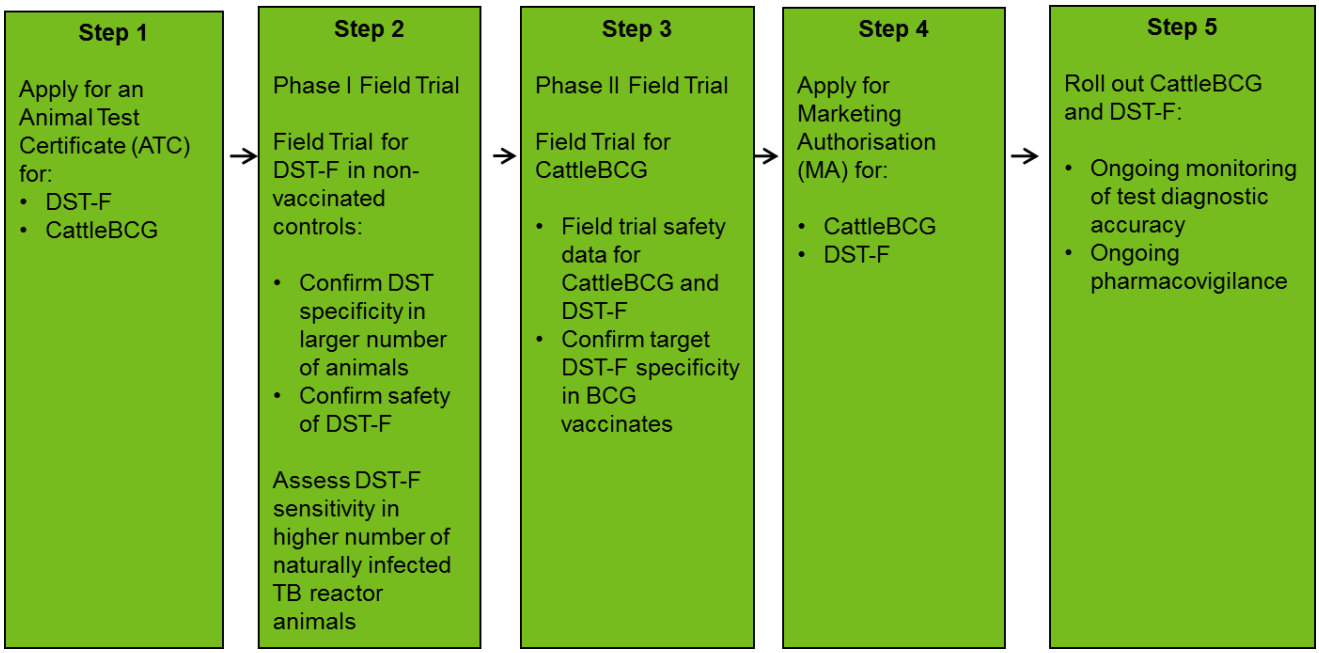


**Fig. 2 Steps to reach UK marketing authorisation for CattleBCG and DST-F**

## Conclusion

APHA is currently undertaking UK Government-funded field trials and additional research, building on several decades of previous work, with the aim of securing an authorised commercial BCG TB vaccine with a complementary DIVA test.

The combination of BCG vaccination and a companion DIVA skin test offer the prospect of an important additional tool in the global efforts to control and eradicate bovine tuberculosis.

The UK's aspiration is for international recognition of these additional tools via future amendments to the OIE Manual of Diagnostic Tests and Vaccines for Terrestrial Animals and the OIE Terrestrial Animal Health Code.

APHA stands ready to support the OIE in this endeavour.

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