

Report of the Meeting of WOAHA's *Ad hoc* Group on Replacement of the International Standard for Bovine and Avian Tuberculin

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1. Background

The current International Standard Bovine Tuberculin (also referred to as “bovine international standard” or “BIS” or “ISBT”) was established in 1986 by the WHO Expert Committee on Biological Standardization. It was originally produced in 1983 by the Central Diergeneeskundig Instituut, The Netherlands. It was evaluated on behalf of WHO by the Central Veterinary Laboratory (CVL), Weybridge, UK, through coordination of an international collaborative study, and was then adopted and designated by WHO. The ISBT is being stored and distributed by the National Institute for Biological Standards and Control (NIBSC), UK, which is a WHO Collaborating Centre and International Laboratory. The ISBT is intended for use in the calibration of ‘in-house standard’ of the bovine purified protein derivative (PPD) tuberculin. In 2015, WOAAH was informed that the ISBT stock is critically low and, based on current demand, it was estimated to be sufficient for until 2026-2028.

The WOAAH *ad hoc* Group (hereafter the Group) on Replacement of the International Standard for Bovine Tuberculin was first convened in [November 2015](#) to replace the current ISBT by establishing a new reference standard (ISBT-2) and the development and evaluation of ‘second generation’ diagnostic tests for bovine tuberculosis.

The Group agreed that any new ISBT should be evaluated and calibrated through a WOAAH-led study guided by an expert panel. This approach was supported by the WHO, which was a member in the Group. The project involved evaluating and calibrating candidate tuberculins against the current ISBT. The Group met in person in [May 2017](#), and [November 2019](#) and to date regular electronic consultations were organised to plan, implement, and analyse the various phases of the project. The Group provided regular updates to the Biological Standard Commission (BSC), and its recommendations are included in the meeting reports published [here](#).

The ISBT has now been removed from the NIBSC catalogue, and its supply has been stopped.

Since 2022, the Group has also been tasked with establishing new international standards for avian tuberculin.

This report is intended to summarise the evolution, results, conclusions, and recommendations of this project from November 2019 to 2025.

2. Overview of the progress: 2015 to 2026.

This section provides a comprehensive account of the planning, methodological evolution, key findings, and discussions arising from scientific experiments conducted by the Group between 2015 and 2025 to identify a suitable replacement for the depleting ISBT. The project involved, NIBSC, 15 national laboratories for tuberculosis, research institutes across the globe, tuberculin manufacturers and multiple testing in guinea pig and cattle experiments.

The project had five milestone phases: donation of bulk material and finalisation of the protocol, candidate selection and preparation of bulk material, preliminary evaluation (PE), International Collaborative Study (ICS), and finally adoption of the new ISBT-2. However, despite extensive evaluation, new experiments were conducted after the completion of the ICS in 2022. In 2026, Candidate B was rejected due to persistent potency shortfalls relative to the ISBT-2 target profile. The section also highlights technical challenges encountered during the experiments, and concludes with considerations for future alternatives, such as Molecularly Defined Tuberculins (MDTs), to strengthen global standardisation of bTB diagnostics.

3. Milestone 1: Donation of bulk material and finalisation of protocol (2015-2017)

In 2015, the Group initially agreed on a protocol for the evaluation of the tuberculin candidate bulk material in line with the WOAAH *Terrestrial Manual* [Chapter 3.1.13](#), which recommended that either live *M.bovis* or inactivated *M.bovis* AN5 could be used for guinea pig sensitisation. The Group agreed that bulk material should be enough to fill 5,000 ampoules of new standard which would be sufficient to meet the estimated demand for the next 20 years. Each ampoule should contain 2 mg of protein having at least 30,000IU/mg verified by a parallel line assay or supported by rigorous post-hoc analysis.

In 2017, the Group noted that the *European Pharmacopoeia (Ph. Eur.)* only recommends using a live *M.bovis* AN5 sensitisation model whereas [Chapter 3.1.13](#) recommended both live and inactive strains sensitisation models for potency determination. The Group had recommended including the inactive strain model as it was logistically and

ethically preferable. However, it acknowledged it also introduces a critical limitation given that the immune responses generated by this method did not adequately replicate those observed in naturally sensitised cattle, thereby reducing assay comparability and the reliability of potency estimates. Despite the above, the Group noted there is additional value in conducting ICS using both live and inactivated *M.bovis* AN5, as it would generate data to determine whether sensitisation with live *M.bovis* AN5 could be replaced by an inactivated *M.bovis* AN5. Nevertheless, in the interest of standardising potency testing, the Group advised using a standardised inactivated sensitising reagent. The Group recommended sensitising guinea pigs in the preliminary evaluation with inactive *M.bovis* AN5, where as for the large-scale ICS, both live and inactive *M.bovis* AN5 strains should be used. The revised protocol ensured compliance with *Ph. Eur.* The protocol was endorsed by BSC in September 2017.

4. Milestone 2: Candidate selection and preparation of bulk material (2015-2017)

In 2015, WOAHA launched a global call for donations of tuberculin candidates and in 2017, the Group evaluated the candidates following a rigorous review of manufacturer dossiers against predefined selection criteria. Three candidates expressed interest in the donation. However, only two candidates (hereinafter referred to as Candidate A and Candidate B) provided sufficient information to the Group. Both Candidates demonstrated compliance with the selection criteria, provided detailed manufacturing protocols and sufficient information on quality control systems, and had a long-standing history of supplying tuberculin for national tuberculosis control programmes. They used *M.bovis* with certified seed lot systems, ensuring traceability and continuity. They could produce sufficient bulk material for 5,000–6,000 ampoules per batch, meeting uniformity requirements. The stated potency of Candidate A was approximately 23,000 IU/mg, and that of Candidate B was 34,000 IU/mg, both within the acceptable range i.e., 50-200% relative to ISBT potency. Therefore, the Group recommended shortlisting Candidate A and Candidate B for lyophilisation / freeze drying of the donated bulk material at NIBSC for preparing it for subsequent preliminary evaluation and international studies.

For more information on the selection criteria for shortlisting the candidate bulk material, the technical qualities of the donated bulk material, and the protocol for the studies, refer to the Group's meeting held in [May 2017](#), and report of the [September 2017](#) BSC.

5. Milestone 3: Preliminary evaluation (PE) (2017-2018)

Two WOAHA Reference Laboratories for bovine tuberculosis (i.e., SENASA and ANSES, France) undertook a **PE** of the candidate tuberculin using a small 'test fill' prepared by NIBSC. The PE was performed in guinea pigs sensitised with inactivated *M.bovis* AN5 strain, ISBT, and with pre-lyophilised and freeze-dried preparations of the two candidate tuberculin. The evaluation was conducted to assess the impact of the lyophilisation process on potency, its specificity, suitability of the candidate for the next phase of international studies. In addition to the above, the PE also included an evaluation of a standardised formulation of heat-inactivated *M.bovis* in mineral oil for potential use as an alternative to *live M.bovis* AN5 for sensitisation of guinea pigs. The results of the PE were satisfactory, and the candidates were recommended for the ICS in 2018.

6. Milestone 4: International Collaborative Study (ICS) (2018-2022)

The ICS was initiated to calibrate candidate tuberculins (i.e., Candidate A and Candidate B) against the current ISBT using harmonised protocols across multiple laboratories. The study included guinea pig assays using the heat-inactivated *M.bovis* AN5 strain, specificity testing with *Mycobacterium avium* to confirm low cross-reactivity, and cattle fitness-for-purpose assays in naturally infected and experimentally infected animals using the comparative cervical test and stability testing to monitor potency retention on long-term storage. The studies concluded in 2019, and based on these results, the Group proposed Candidate B as the preferred replacement for ISBT, as its biological and physicochemical activities were closest to those of ISBT. The Group had recommended that Candidate B be conditionally designated as ISBT-2, subject to successful completion of two additional studies in cattle and stability/shelf-life assessments. Candidate A was recommended for retention as a potential calibrated reference standard for future potency assays. Details regarding these studies can be found in the [November 2019](#) Group meeting report.

However, in 2020, the Group was advised to revise the protocol to include an alternate standard, its methodology, data analysis, and interpretation, and then reassess the potency of Candidate B. It was also advised to include another PPD standard other than the current ISBT should be to mitigate concerns regarding degradation which could lead to variability in potency of the ISBT due to long-term storage. Additionally, it was recommended to

exclude heat-inactivated guinea pig assays, field reactor cattle trials or ring trials as it may not be useful for assigning potency to ISBT-2.

At the meeting of the BSC in [February 2021](#), the Group informed about the revision of the protocol and a new set of experiments was proposed to be conducted based on updated methodology at the WOA reference laboratory for *Mycobacterium tuberculosis complex* (SENASA), Argentina. These trials were completed in 2022, and the Group provided the results of the experiment to the BSC at its [February 2022](#) meeting. The Group reported that potency trials conducted at SENASA using the revised protocol, including its in-house standard as an alternate standard, showed that Candidate B had lower potency than expected. Its relative activity is between 60–70% ($\approx 21,000$ IU/mg), below the agreed minimum target of 30,000 IU/mg. The Group recommended investigating three probable causes for these low potency estimates: degradation of ISBT, precipitation (protein aggregation) of ISBT upon reconstitution, and reduced pathogenicity of the locally employed *M.bovis* AN5 strain.

To address these concerns, the Group recommended that the manufacturer of Candidate B should retest it alongside ISBT and its in-house standard at its facilities. The Group also requests that the potency tests for Candidate B be replicated at another location to confirm SENASA's previous findings and eliminate potential laboratory-specific variability. The UK Health Security Agency (UKHSA) joined the project to conduct the experiments, given their expertise in guinea pig sensitisation assays and their capacity to apply rigorous analytical methods under controlled conditions. Additionally, the Group advised that protein aggregation in both ISBT and Candidate B should be assessed using a particle analyser before injection into guinea pigs, as aggregation could significantly affect potency estimates and explain inconsistencies observed in earlier trials.

7. Post-ICS Assays (2022-2025)

Result of experiments performed by UKHSA and manufacturer (2022–2024)

Based on the Group's recommendations, two separate institutes conducted experiments between 2022 and 2024.

The manufacturer of Candidate B completed the guinea pig assay in their facility using their in-house standard, Candidate B, and the ISBT. The experiment reported good replicability and low variability. However, the potency estimates were significantly below expectations of its Candidate B, ISBT and its in-house standard. These results could not explain the reduced potency of Candidate B and further underscored its continued uncertainty, reinforcing its unsuitability as an ISBT-2.

Similarly, UKHSA conducted a series of guinea pig potency assays using the ISBT, Candidate B, and an in-house standard provided by the manufacturer of Candidate B. The ISBT and Candidate B vials were assessed for precipitation using a particle analyser before use to mitigate the risk of deteriorated ISBT or Candidate B. Throughout these experiments, additional refinements to the protocol were introduced to reduce assay variability and improve infective dose accuracy. This included optimising the inoculation volume from 200 μ L to 50 μ L and extending the interval between sensitisation and PPD administration to 5 weeks to enhance immune responses.

The Group reviewed all results and presented its assessment at the BSC meeting in [September 2025](#). Both the BSC and the Group acknowledged that, despite these efforts, the outcomes of these experiments remained inconsistent, primarily due to weak dose–response relationships, high variability in skin test measurements, differences in immune responses among animals, and a lack of reproducibility across studies and replicates in the infective dose. Based on these limitations, it was not possible to reliably determine the potency of Candidate B, despite repeated protocol refinements and testing efforts.

Results of experiments performed by a private manufacturer (2025)

The Group had recommended conducting one final round of blinded guinea pig assays using the same protocol but implemented by a private tuberculin manufacturer (different from the provider of Candidate B), as they have experience performing these assays at a commercial scale and the likelihood of human error would be minimal, with accurate performance of the assays. Since blinding was an essential step to eliminate unconscious bias in interpreting skin test reactions, one of the experts from the Group visited the manufacturer's premises to conduct the animal readings.

During discussions, the manufacturer sought clarification on the agreed protocol, as the original design was intended for three PPDs (ISBT, an alternate or in-house standard, and Candidate B). In contrast, the manufacturer planned to evaluate only ISBT and Candidate B. The Group reviewed the manufacturer's protocol, which uses four two-fold dilutions (1/250, 1/500, 1/1000, 1/2000 IU) and eight injection sites (four per flank). This approach was considered optimal and analytically superior to the Group's earlier protocol of three five-fold dilutions (1/150, 1/750, 1/3750 IU), which had been adopted due to constraints on number of guinea pigs and the number of PPDs used. The Group noted that the manufacturer protocol of using eight-by-eight Latin square design with four dilutions would provide greater statistical power and minimise experimental bias. The Group thus agreed that they should proceed using its own protocol.

The manufacturer completed the final blinded trials between October and November 2025 and the Group analysed the trial data¹. The potency was estimated using parallel line analysis in COMBISTAT and assigned Candidate B potency between 18,000-19,116 IU/mg, which is below the minimum requirement of 30,000 IU/mg specified in the target profile. The results were consistent from all the assays. The Group concluded that Candidate B showed lower-than-expected potency, likely due to the formulation or stability issues and cautioned that, as ISBT is already has a poorly defined antigen composition, standardising against a lower-potency product would compromise global tuberculin standardisation, reducing comparability of PPD results across laboratories and batches. Consequently, the Group recommended rejecting Candidate B as a replacement for ISBT and that WOAAH restart the process to identify a new candidate for the donation of bulk material.

8. Discussion on alternative approaches to PPD-based testing

The Group also discussed the value of identifying an alternative approach for PPD-based assays. It noted that [recent development](#) in molecularly defined tuberculin (MDT) had indicated comparable sensitivity and specificity to traditional PPD-based skin tests in experimentally infected cattle. MDTs are well-defined antigens that can help reduce within-batch variability, improve standardisation across regions, and demonstrate high sensitivity across various diagnostic platforms. Most importantly, MDT could eliminate the need for complex, biological, animal-based models and allow for more robust quality control methods. The Group considered this opinion and highlighted the need to eventually transition from PPD-based international standards to MDT-based standards, irrespective of the outcome of upcoming ISBT assays. However, the Group acknowledged that MDT is a recent scientific development and that large-scale production would require significant time. Other challenges include limited field validation, potential sensitivity issues compared to PPD, regulatory and logistical complexities, as well as the need for significant R&D investment. The Group recommended continuing the effort to identify new Candidate and relaunch the process using the same technical criteria used for the first call for donation. At the same time, WOAAH should gradually advance the regulatory and scientific experiments needed to accept MDT as a long-term solution.

The Group suggested that WOAAH should open a call for manufacturers to encourage the development of a commercially available MDT antigen as a diagnostic tool for bovine tuberculosis. The WOAAH diagnostic test validation pathway, as outlined in the WOAAH *Terrestrial Manual*, provides the process for incorporating the MDT in its standards. WOAAH could coordinate this effort through its Reference laboratories to ensure scientific rigour and credibility. The Group agreed to develop a concept paper on the process required to establish MDT as an international reference standard for consideration for adoption into the WOAAH standards. At its September 2025 meeting, the BSC acknowledged the concerns and agreed to review the Group's roadmap for MDT development. They cautioned that the Group should consider that, while MDT shows promise as an alternative to traditional biological PPD (i.e., the ISBT), its sensitivity is lower, whereas specificity is higher than PPDs in naturally infected animals. It needs information on the regulatory, scientific, and fieldwork required in different epidemiological settings to establish MDT as an alternative to ISBT in the future.

9. Any other business

.../Annexes

¹ Data from the trial could be provided upon request to the WOAAH Secretariat

Annex 1. List of Participants

MEETING OF THE AD HOC GROUP ON REPLACEMENT OF THE INTERNATIONAL STANDARD OF BOVINE AND AVIAN TUBERCULIN

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