



**WHO Reference Reagent  
Anti-Mycoplasma gallisepticum Serum  
NIBSC code: MGDS  
Instructions for use  
(Version 8.0, Dated 24/01/2014)**

### 1. INTENDED USE

This material was prepared in chickens inoculated with the S6 strain of Mycoplasma gallisepticum at the Veterinary Laboratories Agency (VLA) in Weybridge (UK). It can be used to standardise agglutination assays, the Haema agglutination inhibition (HI) assay, the rapid agglutination assay and the slow agglutination assay. The sensitivity/potency of the serum varies depending on the choice of antigens. For HI antigens, 25-2.7 IU per ml gave 50% agglutination, for the rapid agglutination antigens this is 2.5 IU and for the slow agglutination antigens this 12.5-25 IU per ml.

### 2. CAUTION

**This preparation is not for administration to humans or animals in the human food chain.**

Chicken source material. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

### 3. UNITAGE

Each ampoule contains 1000 IU. Assigned content of vial valid at time of manufacture – no information on long term stability.

### 4. CONTENTS

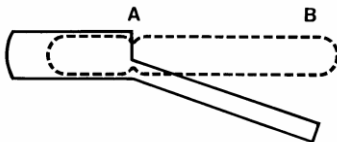
Country of origin of biological material: United Kingdom.  
See attached insert from the VLA for details.

### 5. STORAGE

Reconstituted material should be stored at 4°C for upto 6 months.  
**Please note: because of the inherent stability of lyophilized material, NIBSC may ship these materials at ambient temperature.**

### 6. DIRECTIONS FOR OPENING

Tap the ampoule gently to collect the material at the bottom (labelled) end. Ensure ampoule is scored all round at the narrow part of the neck, with a diamond or tungsten carbide tipped glass knife file or other suitable implement before attempting to open. Place the ampoule in the ampoule opener, positioning the score at position 'A'; shown in the diagram below. Surround the ampoule with cloth or layers of tissue paper. Grip the ampoule and holder in the hand and squeeze at point 'B'. The ampoule will snap open. Take care to avoid cuts and projectile glass fragments that enter eyes. Take care that no material is lost from the ampoule and that no glass falls into the ampoule.



Side view of ampoule opening device containing an ampoule positioned ready to open. 'A' is the score mark and 'B' the point of applied pressure.

### 7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution.

### 8. STABILITY

It is the policy of WHO not to assign an expiry date to their international reference materials. They remain valid with the assigned potency and status until withdrawn or amended.

Reference materials are held at NIBSC within assured, temperature-controlled storage facilities. Reference Materials should be stored on receipt as indicated on the label. For information specific to a particular biological standard, contact the Technical Information Officer or, where known, the appropriate NIBSC scientist.

In addition, once reconstituted, diluted or aliquoted, users should determine the stability of the material according to their own method of preparation, storage and use.

NIBSC follows the policy of WHO with respect to its reference materials.

Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

### 9. REFERENCES

Stewart, DL et al., Bull WHO 1971; 45:219

### 10. ACKNOWLEDGEMENTS

N/A.

### 11. FURTHER INFORMATION

Further information can be obtained as follows;  
This material: [enquiries@nibsc.org](mailto:enquiries@nibsc.org)  
WHO Biological Standards:  
<http://www.who.int/biologicals/en/>  
JCTLM Higher order reference materials:  
<http://www.bipm.org/en/committees/jc/jctlm/>  
Derivation of International Units:  
[http://www.nibsc.org/standardisation/international\\_standards.aspx](http://www.nibsc.org/standardisation/international_standards.aspx)  
Ordering standards from NIBSC:  
<http://www.nibsc.org/products/ordering.aspx>  
NIBSC Terms & Conditions:  
[http://www.nibsc.org/terms\\_and\\_conditions.aspx](http://www.nibsc.org/terms_and_conditions.aspx)

### 12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to [enquiries@nibsc.org](mailto:enquiries@nibsc.org)

### 13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.



**14. MATERIAL SAFETY SHEET**

Physical and Chemical properties	
<b>Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008:</b> Not applicable or not	Corrosive: No
	Physical appearance: Freeze-dried powder
Stable: Yes	Oxidising: No
Hygroscopic: No	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains chicken serum	
Toxicological properties	
Effects of inhalation: Not established, avoid inhalation	
Effects of ingestion: Not established, avoid ingestion	
Effects of skin absorption: Not established, avoid contact with skin	
Suggested First Aid	
Inhalation: Seek medical advice	
Ingestion: Seek medical advice	
Contact with eyes: Wash with copious amounts of water. Seek medical advice	
Contact with skin: Wash thoroughly with water.	
Action on Spillage and Method of Disposal	
Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.	

**15. LIABILITY AND LOSS**

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at [http://www.nibsc.org/About\\_Us/Terms\\_and\\_Conditions.aspx](http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx) or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

**16. INFORMATION FOR CUSTOMS USE ONLY**

<b>Country of origin for customs purposes*:</b> United Kingdom * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
<b>Net weight:</b> 0.06 g
<b>Toxicity Statement:</b> Non-toxic
<b>Veterinary certificate or other statement</b> if applicable. <b>Attached:</b> No

**17. CERTIFICATE OF ANALYSIS**

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards [http://www.who.int/bloodproducts/publications/TRS932Annex2\\_Inter\\_biol\\_standardsrev2004.pdf](http://www.who.int/bloodproducts/publications/TRS932Annex2_Inter_biol_standardsrev2004.pdf) (revised 2004). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.



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**INTERNATIONAL REFERENCE PREPARATION OF  
ANTI-MYCOPLASMA GALLISEPTICUM SERUM (MgDS)**

**Description**

The International Reference Preparation of Anti-*Mycoplasma gallisepticum* Serum was established in 1969. It is intended for standardizing the haemagglutination-inhibition (HI) test and agglutination test for *Mycoplasma gallisepticum*.

It was prepared by inoculating 10-week-old *M. gallisepticum*-free chickens intranasally, intraocularly and/or into the intranasal sinus with a 24 hour broth culture of an S6 strain of *M. gallisepticum* (D.V. Zander, 1961. Avian Dis. 5, 155) using 0.25ml of culture per bird. The birds were bled from the heart 6 weeks later. The serum was pooled, filtered, preserved with 1/10,000 merthiolate and freeze-dried in neutral-glass ampoules in 1ml aliquots; the ampoules were filled with dry nitrogen and sealed at approximately atmospheric pressure.

The average weight of dried material per ampoule is 0.0556g with a standard deviation of 0.7%.

Full details of the establishment of the preparation have been described (Stewart, D.L., Davidson, I., Hebert, C.N. and Frerichs, C.C. (1971) Bull Wild Hlt Org 45 219).

**International Unit**

The International Unit is defined as the activity contained in 0.0556mg of the International Reference Preparation.

Each ampoule contains 1000 International Units.

**Distribution**

The International Reference Preparation is distributed by the International Laboratory for Biological Standards, Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Addlestone, Surrey, England, on behalf of the World Health Organisation. It is available free of charge in limited amounts. If a laboratory needs more than one sample every six months, it is expected to prepare its own reference preparation and to calibrate it against the International Reference Preparation.

**Reconstitution of the International Reference Preparation**

The preparation should be reconstituted immediately before it is to be used.

Ampoules may be opened by scoring with a small saw specifically designed for the purpose, or a hard mineral edge, for approximately one third of the circumference. Application of a piece of red hot glass rod to this scratch will give a clean line of fracture.

If the scoring is made firmly and the glass rod is hot enough, it is possible to produce a fine crack without disturbing the ampoule top until needed. Then slight pressure will complete the separation.

*An Executive Agency of the  
Ministry of Agriculture,  
Fisheries and Food*



The freeze-dried material in each ampoule may be reconstituted in any convenient volume of a suitable diluent.

Care should be taken to ensure that the entire contents of the ampoule are completely resuspended. This can be achieved by suspending the bulk of the contents of the ampoule in some of the fluid, and using the remainder of the diluent to rinse out the ampoule three times.

A concentrated solution of the Reference Preparation, in physiological saline, e.g. if the contents of one ampoule are resuspended in 10ml, may be stored at about 4°C for up to six months without detectable loss of potency. A suitable preservative may be added if desired.

### **National and Laboratory Reference Preparations**

National and laboratory reference preparations should be prepared in a stable form. This may be achieved by freeze-drying aliquots in neutral-glass ampoules and sealing them in an oxygen-free atmosphere by fusion of the glass. The ampoules should be stored in the dark at a low temperature, e.g. -20°C.

The potency of such a reference preparation relative to that of the International Reference Preparation should be determined by performing a series of comparative tests; these should be carried out by each of the test methods (rapid plate agglutination, slow agglutination and/or HI) for which it is intended to use the reference preparation. After determining the titres of the two sera approximately the tests should be repeated using dilutions closely spaced around the expected end point. The dilutions should cover the range from complete agglutination or inhibition to no agglutination or inhibition.

The number of International Units per ampoule of the reference preparation is calculated from its titre relative to that of the International Reference Preparation. This calculation should be based on a series of at least three tests.

It is suggested that the potency of a national or laboratory reference preparation should be checked against that of a fresh sample of the International Reference Preparation about once a year.

The International Laboratory for Biological Standards at Weybridge is willing to advise and assist laboratories in providing their own standards.

### **Routine Agglutination Tests and Haemagglutination-Inhibition Tests**

The reference preparation can be used to standardize agglutination tests and HI tests in the following ways:

#### **1. Standardization of Antigens**

##### **HI Test Antigen**

The concentration of an antigen is first adjusted approximately by measuring the antigen's haemagglutinating activity. The 50% HA unit is defined as that amount of antigen giving 50% haemagglutination in the test system employed. The final adjustment should be made by performing a test with a reference preparation calibrated in International Units (I.U.) and adjusting the concentration of antigen to give a pre-determined titre with that preparation.

The results of the collaborative assay by which the I.R.P. was established may be taken as a guide to the optimum concentration of antigen. The HI antigens gave 50% inhibition with a serum containing between 2.7 and 25 I.U./ml.

##### **Rapid Agglutination Test Antigen**

The sensitivity of an antigen is determined with respect to the International Reference Preparation by the following method. An equal volume of doubling dilution of the International Reference Preparation is added to 0.05ml of antigen. A positive reaction is recognised by the formation of coloured follicles





and the clearing of the suspended medium. Agglutination must be clearly visible with the dilution containing 2.5 I.U. It must appear in 30 seconds following mixing and, at the end of 2 minutes, comprise 50% of the cell suspension.

#### **Slow Agglutination Test**

Collaborative assay results indicated that an antigen giving 50% agglutination with a serum containing between 12.5 and 25 I.U./ml is suitable for this test.

As well as standardizing antigens quantitatively as described above, it is advisable to check each batch of antigen qualitatively by testing it against a collection of known positive and negative sera from both fowls and turkeys to show that it gives the expected result in each case.