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NIBSC

The importance of physical reference materials in assigning biological activity and improving value assignment in International Standardisation.

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INTRODUCTION

Biological medicines are highly complex and despite huge progress over the past 25 years in biophysical methods full characterization of the biological activity of all but the smallest proteins/peptides is not possible solely in terms of SI-traceability.

The harmonization of biological activity in biotherapeutics and vaccines has been a priority of the World Health Organisation (WHO) for many years and NIBSC has played a central part in achieving that harmonization, acting as a main Collaborating Centre, Reference Material manufacturer and Custodian. Over 400 reference materials are available—see ***www.nibsc.org***

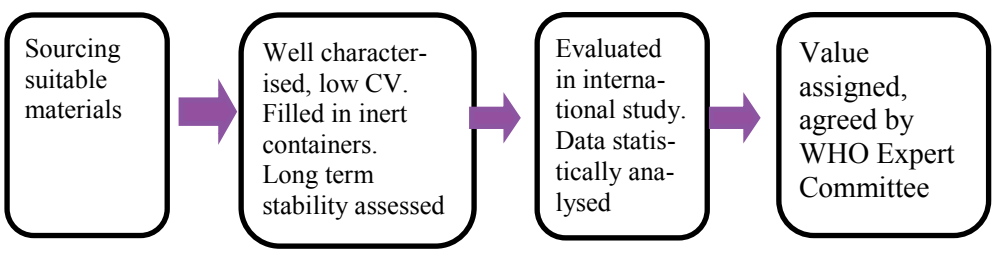
This achievement comes through the preparation and distribution of stable biological reference materials which act as a "gold standard" for assay purposes.

Fig 1: Some International Standards— past (left) and present (right)

PATHWAY TO DEVELOPING AN INTERNATIONAL STANDARD

The preparation of International reference preparations has been described both in guidelines (1) and also the manufacturing processes adopted at NIBSC reviewed (2,3). In brief, these rely upon:

- Well characterised (though not necessarily pure) materials
- Accurate dispensing of fill to deliver homogeneity
- Stable and inert containers—glass ampoules being the preferred format
- Long term stability—through appropriate formulation and freeze drying
- International collaborative evaluation
- Statistical analysis and consensus value assignment



IMPORTANCE OF USING INTERNATIONAL UNITS

WHO define biologicals as medicines whose activities are not fully characterizable by physicochemical means alone. Therefore in these cases the potency is not defined by a SI-traceable value but by an arbitrary unit defined by an International Standard reference material.

For instance, NIBSC has developed an International Standard for the TNFα binding activity of the therapeutic Mab infliximab, used to treat inflammatory disease such as rheumatoid arthritis. In this study (Metcalfe et al 2018) (4) lyophilized preparations of infliximab that were subjected to an international collaborative study. This study consisted of 26 participants based in 15 countries with laboratories from regulatory agencies, official medicine control institutions, contract research organisations, academia and the pharmaceutical sector . A value of 500 IU/ampoule was assigned following the study**.** This preparation will allow comparison of anti-TNFα Mabs in terms of their bioactivity (Fig 2).

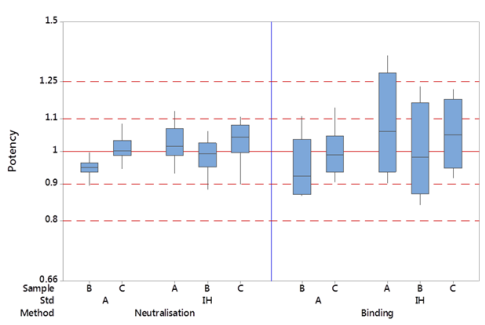


Fig 2: Infliximab activity. Assay against a common reference standard lowered inter laboratory variation and improved inter – laboratory agreement. See (4).

World Health Organization

HOMOGENEITY OF ACROSS –BATCH POTENCY

As the Unit of Activity relates to the physical reference material ampoule there is no uncertainty of measurement assigned to the value. Instead the uncertainty resides in the variability of the fill and so a precise fill is demanded (filling weight <0.25% for aqueous materials) ensuring that all containers within a batch have defined potency within a tightly controlled range of potency.

IMPORTANCE OF REFERENCE MATERIAL CHOICE

Material choice is a critical parameter in delivering good harmonization (Fig 3). Fryer et al showed that a synthetic plasmid material gave poorer harmonization for measurements of CMV by participant laboratories whereas the live virus RM gave much better consensus (5).

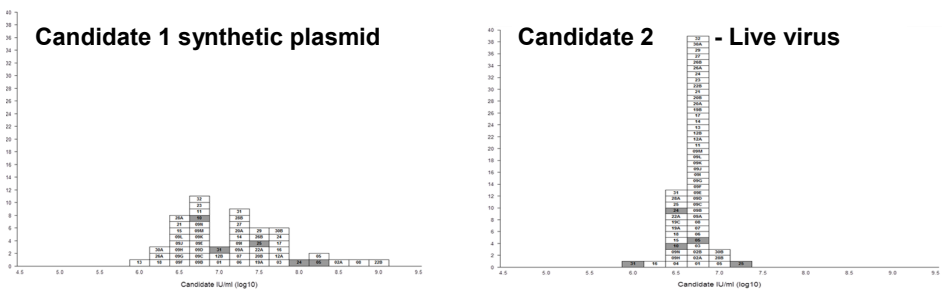
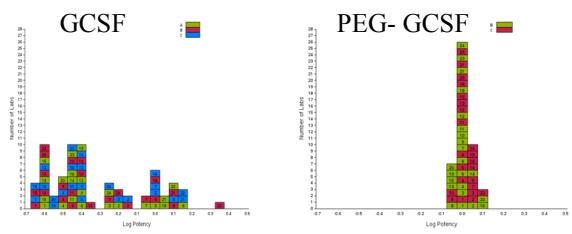


Fig 3: Improved harmonization of collaborative laboratory data when reference material type matched sample

Fig 4: Harmonization of PEG-GCSF test sample results when compared against a GCSF IS (09/136) (LHS) & the candidate PEG-GCSF IS



This is also seen for biotherapeutics (Fig 4) such as PEG-GCSF where better agreement in the relative potency estimates from collaborative laboratories was seen when using a reference material which included the PEG moiety (6).

CONCLUSIONS

For the assignment of biological activity the direct use of a physical reference preparation remains the best solution

The important role remains for the arbitrary International Unit of bioactivity in determination of biological potency

We have illustrated how such standards must closely match their test subject in order to give optimal harmonization

The processing properties define the homogeneity and are critical to assure harmonization

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