

Preparation of reference standards final draft. Adopted by the 55th meeting of the WHO Expert Committee on Biological Standardization, 15-18 November 2004. A definitive version of this document, which will differ in editorial detail but not scientific content, will be published in the WHO Technical Report Series

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**WORLD HEALTH ORGANIZATION
ORGANISATION MONDIALE DE LA SANTE**

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**RECOMMENDATIONS FOR THE PREPARATION,
CHARACTERIZATION AND ESTABLISHMENT OF
INTERNATIONAL AND OTHER BIOLOGICAL REFERENCE
STANDARDS**

(Revised 2004)

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DRAFT

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60 **INTRODUCTION**

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62 A core function of WHO, set out in its Constitution (Article 2), is to "develop, establish

63 and promote international standards with respect to food, biological, pharmaceutical and

64 similar products" as well as "to standardize diagnostic procedures as necessary". This

65 responsibility is discharged in part by establishment of biological reference standards that

66 form the basis of regulation and clinical dosing for biological medicines and also for

67 regulation of *in vitro* diagnostic devices. The process whereby such international biological

68 reference standards are established and the technical specifications to which they comply are

69 set out in this guidance document, which is intended to be scientific and advisory in nature.

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72 The provision of international biological reference standards makes a critically
73 important contribution to recurring high standards of efficacy, quality, purity and safety of
74 very many biological medicines used worldwide in the prevention, treatment or diagnosis of
75 disease or conditions. Their use supports the application of the many biological and
76 immunological assays used in the standardization and control of a wide range of biologicals
77 including therapeutics, blood-derived products, vaccines and immunological products of
78 traditional types as well as those derived from modern biotechnological approaches. They
79 also have important applications in the standardization of materials and approaches used in
80 medical diagnostics such as diagnosing disease, monitoring therapy, blood safety, and public
81 health applications (eg. monitoring immune status, screening for disease or susceptibility) or
82 otherwise characterizing biological material from individuals.

83

84

85 WHO biological reference standards are widely used in the development, evaluation,
86 standardization and control of products in industry, by regulatory authorities and also in
87 biological research in academia and scientific organizations. They play a vital role in
88 facilitating the transfer of laboratory science into worldwide clinical practice and the
89 development of safe and effective biologicals.

90

91 There are special considerations and challenges which apply to the production and
92 quality evaluation of biologicals, including the inherent variability of biological systems,
93 variability of biological and immunological assays, and the potential for microbial
94 contamination. The availability of WHO reference standards has made a major contribution
95 to progress in the development and use of biologicals and in addressing these challenges.

96

97 In particular the reference standards have an essential role in the development of
98 internationally agreed systems for measurement of biological and immunological activities
99 residing in biological products. There is a wide variety of potential types of measurements:
100 for example biological activity, immunological activity, quantity, biotypes and genetic types.
101 In addition, for each measurement type, there are numerous variations of methodologies and
102 reagents. Therefore, the purpose of the reference standards is to facilitate standardized
103 characterization of biological samples, no matter what type of measurement or method is

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104 used. Many WHO biological reference standards are designated as International Standards
105 (IS) and provide the unique physical basis for the definition of International Units (IUs) of
106 biological and/or immunological activity. Their use enables the achievement of consistency
107 in the measurement of key attributes of biologicals, for example biological potency or
108 immunological activity and, thus, the development of internationally agreed criteria for
109 acceptability and standardization and control of products. It also provides the basis for the
110 comparability of data from different sources in relation to specific products. Assays for
111 markers of immunity (e.g. to infectious agents) are often defined in terms of agreed IUs of
112 antibodies, providing a basis for an international consensus on the measurement of the
113 immunological status of individuals or populations following vaccination or infection.

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116 Some WHO biological reference standards do not carry the designation of ISs, but are
117 nevertheless of great value in the standardization of assays applied to biological products and
118 diagnostic materials.

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121 The timely development of new reference materials and standards is a critically
122 important aspect of harnessing new scientific developments for safe and effective application
123 in the form of safe and effective biologicals and securing improved world health.

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126 This document provides an updated set of recommendations in relation to the
127 development, evaluation, establishment and use of WHO biological reference materials.
128 The WHO Guidelines for the Preparation and Establishment of Reference standards for
129 Biological Substances were first published in 1978 (1). The Guidelines were revised in 1986
130 (2) following decisions by the WHO Expert Committee on Biological Standardization to
131 simplify the nomenclature of international biological reference standards (3) and that
132 reference standards of human origin should be tested for evidence of possible contamination
133 with human immunodeficiency viruses and hepatitis B virus (4). The Guidelines were revised
134 again in 1990 (5) when a section was added on information to be provided in support of
135 requests for adoption by the WHO Expert Committee on Biological Standardization of
international biological reference standards.

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136 A number of developments have occurred since 1990. Partly because of scientific and
137 technical advances, the range of materials classified as biological substances has altered:
138 many older biologicals can be appropriately characterized by chemical and physical means
139 and their WHO biological reference standards have been discontinued, while new groups of
140 biological substances have been developed.

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Antibiotics came within the range of substances considered by WHO as biologicals at the time of their development though now, for most antibiotic preparations, physico-chemical testing, rather than biological testing, is accepted.

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On the other hand new groups of substances have been developed through advances in molecular biology. Biological reference standards are still needed when such materials are subjected to biological or immunological assay.

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156 The need has been recognized for prompt availability of some reference standards without the

157 rigorous characterization and testing of international biological standards, leading to a new

158 group of WHO Reference Reagents which may act as interim standards (6). A priority

159 setting process for developing WHO biological reference standards has been published (7).

160 The science of reference standard preparation and characterization has continued to evolve

161 and the extent to which principles for the characterization of reference standards in certain

162 fields (8) can be applied to biological reference standards as a whole has been debated.

163 Consequently, WHO has worked with the scientific community, national regulatory

164 authorities, other standards setting bodies and users through a series of consultations (8a, 8b,

165 8c,8d,8e) to review the scientific basis of characterization of biological reference standards.

166 As a result, the concepts used by WHO for biological standardization are re-affirmed as

167 appropriate to ensure the continued usefulness of this class of reference standards. During the

168 consultation process it was however recognised that improved clarity in explaining the

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169 rationale for the principles used by WHO in biological standardization would be of benefit.

170 This updated version of the Guidelines reflects these and other changes.

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172 These Recommendations are in three parts:

173

174 • General Considerations address the scientific basis of biological standardization and
175 the principles applied to WHO International Standards.

176 • Part A addresses the background to the need for an international biological reference
177 standard, general considerations about procurement and characterization of suitable

178 material, factors to be taken into account in preparing a batch of a candidate reference

179 standard and assessing its suitability, the testing and collaborative assay of the batch, and

180 the information to be provided to WHO so that appropriate reference standards can be

181 established by the WHO Expert Committee on Biological Standardization (ECBS). A new

182 section has been introduced on quality assurance considerations.

183 • Part B provides advice and guidance to regional and national control authorities on the

184 preparation and establishment of secondary biological reference standards. Such

185 materials may be assigned values in International Units (IUs) by assay against the

186 corresponding WHO reference standard.

187

188 The parts of each section printed in indented text are comments for additional guidance

189 and are intended to provide further explanation of the main text.

190

191 **GENERAL CONSIDERATIONS**

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193

194 WHO biological reference standards are comprised of materials of complex

195 composition. that require biological or immunological assay for appropriate characterization.

196 The biological or immunological assays used are usually comparative rather than absolute, and

197 the reference standard is critical in defining the qualitative nature and the relative magnitude of

198 the biological or immunological response. The published catalogue of WHO biological

199 reference standards includes over 300 materials and is updated each time materials are added

200 or removed from the list (8). Definitions used in the context of this document, are given in

201 part A, section 1.2.

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203 The set of principles used by WHO for biological standardization are:

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205 1 that the reference standard should be assigned a value in arbitrary rather than absolute
206 units, but there can be exceptions, where justified

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208 2 that the unit is defined by a reference standard with a physical existence

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210 3 that in the establishment of the standard a variety of methods is usually used and that
211 the value assignment to the standard, and therefore the definition of the unit, is not
212 necessarily dependant on a specific method of determination

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214 Generally, WHO reference standards are
215 established for analytes where no reference
216 measurement procedure (“reference method”) has
217 been agreed or established. In these cases the
218 principles set out above will apply. Where a
219 reference method has been defined and agreed,
220 then establishment of the standard and value
221 assignment may be specifically based on that
222 method.

223

224 4 that the behaviour of the reference standard should resemble as closely as possible the
225 behaviour of test samples in the assay systems used to test them.

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227 The general principle is that of “like vs
228 like”. Thus whilst it may not be
229 necessary for the standard to be prepared
230 in the same formulation or matrix as test
231 samples, it is necessary that the dose-
232 response characteristics of the standard are
233 the same as those of tests samples. For
234 example, the reference preparation for

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268 and is intended for assay of poliovirus
269 mutants in vaccine preparations (add
270 ref).

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274 These principles derive from shared properties of complex macromolecular analytes :

- 275 - difficulties in unambiguously assigning a value in SI units, even to well-
- 276 characterised proteins
- 277 - the comparative rather than absolute nature of biological and immunological
- 278 test procedures
- 279 - the difficulty in quantitatively defining the analyte in terms of a biological
- 280 response
- 281 - the difficulty of defining reference methods
- 282 - the multi-factorial nature of biological and immunological test methods, where
- 283 both quantitative and qualitative differences in activity may result from
- 284 changes in the properties of the reference standard.

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The implications are two fold for the establishment of biological reference standards; firstly, that an analyte complying with this definition is in fact defined by the reference standard. This is distinct from the situation for some chemical reference standards, which can be fully characterised by physical or chemical methods, where an analyte is defined by a reference method. Secondly, it cannot be proven analytically that, when a biological reference standard is replaced, the new material is identical to the old. The

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301 analyte is essentially re-defined by the
302 new reference standard. This means that
303 the chain of traceability for the user is to
304 the new reference standard. Where a
305 reference standard is assigned an activity
306 expressed in International Units, every
307 effort is made in the collaborative study
308 design to ensure that the IU defined by a
309 replacement reference standard is as
310 similar as possible to the IU defined by
311 the old reference standard so that
312 continuity of the IU is maintained over
313 time. For example, the International Unit
314 of Factor VIII activity in factor VIII
315 concentrate was established in 1970, and
316 the activity represented by this unit has
317 been maintained through seven
318 successive WHO International standards,
319 providing a stable baseline over time to
320 assess and compare the efficacy of factor
321 VIII treatments for Haemophilia.

322
323
324 The relative magnitude of biological
325 responses forms the basis of the
326 comparative procedures in which
327 biological reference standards are used.
328 It is desirable that biological reference
329 preparations are not assigned values in
330 terms of the absolute magnitude of the
331 biological response, since this depends
332 on a variety of conditions. For example,
333 WHO collaborative studies typically

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334 show that an absolute biological
335 response such as a 50% cell culture
336 infectious dose (CCID50) is more
337 variable than expression of results as a
338 relative potency in IU. In a few cases, for
339 historical reasons, some standards are
340 defined in terms of a 'consensus'
341 absolute biological response and are not
342 used for assignment of relative potencies.
343 An example would be the IS for measles
344 vaccine (live) which is assigned a value
345 of 4.4 log₁₀ infectious units per
346 ampoule. It should be noted that this
347 leads to difficulties in maintaining
348 continuity of the assigned unit when it is
349 necessary to replace such a standard.

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353 As a consequence of applying these
354 principles, the activity or potency of a
355 WHO biological reference standard is
356 demonstrated by biological procedures
357 and, where appropriate, is stated in
358 arbitrary International Units. The
359 reference preparation thus defines the
360 numerical value of the unit and also has a
361 role in qualitatively defining what is
362 being measured (the analyte). It is
363 implicit that the unit has no existence
364 other than in relation to the reference
365 preparation that defines it. Thus when
366 stocks of a WHO biological reference

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367 preparation are depleted, it is of high
368 priority that a replacement material be
369 established in its place (Appendix 1).
370 When a replacement standard is
371 established the units defined by the
372 previous standard formally cease to exist.
373 In practice, every effort is made to assign
374 a value to the new reference preparation
375 to preserve as closely as possible the
376 value of the International Unit over time
377 (continuity of the unit). This ensures that
378 users do not experience differences from
379 one year to the next (or one decade to the
380 next) when using values derived from
381 WHO biological reference preparations.
382 A further consequence of the principles
383 given above is that multiple methods,
384 and in particular those methods which
385 are currently in use in the relevant field,
386 are usually used in studies to characterize
387 candidate biological reference standards.
388 This approach embodies the recognition
389 that it is usually not possible to select, on
390 a rational basis, any single assay method
391 from which to predict the biological
392 activity in humans of a preparation.

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396 It is recognized that biological materials may be shown to have different types of
397 biological activity. Thus separate reference preparations may be established for bioassay and
398 immunoassay standards, or, assignment of different types of biological activity to the same
399 reference preparation may occur.

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As an example of establishing separate reference

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preparations, the 1st International Standard (IS) for

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follicle-stimulating hormone, recombinant, for bioassay

404

was established in 1995 with an activity of 138

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IU/ampoule (6) and a different preparation was

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established in 1997 as the 1st IS for follicle-stimulating

407

hormone, recombinant, for immunoassay with an activity

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of 60 IU/ampoule (10). Two reference standards are thus

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available for different uses and users need to ensure that

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the appropriate material is requested, depending on the

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intended use.

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As an example of assigning different types of biological

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activity to the same reference preparation, the 2nd IS for

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low-molecular-weight heparin, established in 2003 (11),

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was assigned activities of 1097 IU of anti-Xa per ampoule

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and 326 IU of anti-IIa per ampoule.

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It is also recognized that some international standards may be used for qualitative rather than quantitative purposes.

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This is particularly the case for some IS materials used

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in the *in vitro* diagnostics area. In such cases, an IS may

424

be established without the assignment of an IU. In some

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cases no assignment of activity may be made or,

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alternatively, units of activity may be assigned in terms

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of a suitable property. For example, the 1st International

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Standard for MAPREC analysis of poliovirus type 3 is

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assigned a content of 0.9% 472-C nucleotide per

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ampoule (add ref).

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432 Previously, a reference standard established without an
433 assigned IU was called an international biological
434 reference reagent (5). However even at the time these
435 two categories were created it was acknowledged that
436 the distinction between the two was not always clear-cut
437 (5). In this revision, the distinction is no longer
438 maintained. It is essential at the outset of any study of a
439 candidate biological reference standard to clearly state if
440 the intended use of the material is for qualitative
441 purposes, since this will significantly influence the study
442 design.

443
444 It may also be necessary to establish a panel of materials
445 as a reference panel to aid evaluation of diagnostic tests.
446 As an example, the ECBS established a reference panel
447 of 10 individual genotypes of human immunodeficiency
448 virus type 1 (HIV-1) to help assess the specificity of
449 nucleic amplification technology based assays for HIV-
450 1. The panel was established as the First International
451 Reference Panel for HIV_1 genotypes and unitages were
452 not assigned to the individual members of the panel
453 (11a).

454
455 The extent to which the general metrological topic of *measurement uncertainty*, as
456 defined in the standard ISO 17511 (8) applies to biological reference standards has been
457 raised in the light of new regulations from one region of the world concerning *in vitro*
458 diagnostic devices. However, where international biological reference standards are to be
459 assigned a value in arbitrary International Units, an uncertainty value is not given.

460
461 As a consequence of defining the IU to the
462 current IS and not formally to any previous IS, an
463 uncertainty value is not given to the assigned IU.

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465 Information on the variability observed during
466 the course of a collaborative study to characterise
467 the preparation is always documented in the
468 collaborative study report, which is available to
469 users. In a multi-method collaborative study,
470 differences (in potency estimates of the material)
471 between methods may be apparent. Moreover,
472 the nature of biological assays means that
473 methods which are nominally the same in reality
474 differ in many features. In the absence of a
475 reference method, assumptions about an
476 underlying true value (of potency of the
477 material), or a probability distribution of values
478 across methods, may not be valid. Summarising
479 all the components of variability observed in a
480 collaborative study by quoting a single
481 uncertainty value may not be helpful. It does not
482 reflect the variability between ampoules of the
483 IS.

484
485 The memoranda accompanying reference
486 standards should contain a statement of the
487 coefficient of variation (CV) of fill of the
488 preparation concerned to reflect ampoule to
489 ampoule variation (11).

490
491 Another issue raised by ISO17511 (8) is the assumption of a metrological
492 “hierarchy”, in which SI units are of a higher metrological order than IU. A strict application
493 would appear to imply that, where possible, procedures reporting SI units should be used to
494 calibrate reference preparations regardless of any other considerations. The ECBS, after
495 consideration of this issue (11), concluded that the choice of unit should reflect, and be based
496 on, the biological and medical as well as the physicochemical information available on a
497 case-by-case basis.

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Many biologicals exist in both active and inactive states, and the clinically relevant form of the analyte may depend on the diagnostic aim. For example, the active state of the placental hormone chorionic gonadotrophin (hCG) is the relevant molecule to measure in the diagnosis of pregnancy, whereas the biologically inactive free beta subunit (hCG-beta) is measured to diagnose choriocarcinoma. Generally, measurement of a biological activity is expressed in IU, whereas measurement of the amount of a protein or of a specific protein structure is expressed in SI. In this case there would be a compelling reason to relate the measurement of hCG to a unit of biological activity, and the measurement of hCG-beta to an SI unit of quantity. Accordingly WHO has established a reference preparation for hCG (currently the fourth International Standard, with an assigned content of 650 IU/ampoule) (add ref) and a reference preparation for hCG-beta (currently the first WHO Reference Reagent for immunoassay of hCG beta subunit, with an assigned content of 0.88 nmol/ampoule) (add ref). The former preparation was assigned a value based on bioassay whereas the latter preparation had been extensively characterized by physicochemical and immunological methods and calibrated in nanomol by amino acid analysis.

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530 Applying these considerations of the properties
531 of biological analytes, and their measurement in
532 the clinical situation, allowed the WHO
533 biological reference standard for hepatitis B
534 surface antigen, assigned a value in arbitrary IU
535 rather than in SI units, to be adopted by the
536 medical devices sector of the European
537 Commission as the standard required for the
538 fulfilment of the so-called Common Technical
539 Specifications (CTS) for in-vitro diagnostic
540 devices. The Common Technical Specification
541 document supporting the European (IVD)
542 Medical Devices Directive 98/79 EC is a legally
543 binding document within the 25 countries of the
544 European Union.

545
546 Where it is appropriate for a WHO biological
547 reference standard to be calibrated in SI units,
548 the principles outlined in ISO 17511 (8) should
549 be followed. This will necessitate the existence
550 and use of an appropriate single reference
551 method and an assignment of uncertainty,
552 derived from calibration data. Such a reference
553 method should not be a biological assay since
554 the factors that affect the results of such assays
555 cannot be fully described. Where they are used
556 SI units assigned to biological reference
557 standards should be derived from and traceable
558 to physico-chemical procedures.

559
560 The decision on the route of characterization to be followed for a WHO biological
561 reference standard must be clearly made at the outset of the study.

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563 The concept of commutability also needs to be addressed. The way in which this is
564 done may vary depending on the field of application. In the *in vitro* diagnostics area the
565 analyte may be a minor component of a complex biological matrix (eg. blood). Matrix
566 effects may have an important effect on the measurement.

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In general terms the concept of commutability seeks to establish the extent to which the reference standard is suitable to serve as a standard for the variety of different samples being assayed. This concept is considered in ISO15194 (add ref) to be an intrinsic property of the standard, and to require description. The way in which this is done may vary according to the intended application.

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In the vaccine field, for example, International Standards for vaccines may be prepared without adjuvant, whereas vaccines preparations usually contain adjuvant. The applicability (or commutability) of the standard to such preparations will need to be established, either in the collaborative study, or by independent validation of assay methods.

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Commutability in the *in vitro* diagnostics field is a consideration of how a reference preparation and samples to be examined compare in different assay methods, and is a property potentially affected by a wide range of factors including matrix (plasma, urine), binding proteins, plasma degradation and molecular variants of the analyte. A number of experimental approaches have been defined to

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596 determine this property, for example, a
597 comparison of the ratio between the results of
598 two procedures for the reference standard and
599 for test samples.. A commutable biological
600 reference standard shows a similar behaviour to
601 routine samples when different measurement
602 procedures are applied. Generic specifications
603 for similarity are difficult to formulate and are
604 addressed on a case-by-case basis. Inclusion of
605 real or surrogate clinical samples in the
606 collaborative study may be a useful approach to
607 enable evaluation of commutability. However it
608 should be noted that it can only be stated for the
609 methods and samples studied, and a more
610 extensive evaluation of commutability may
611 require additional studies outside the WHO
612 collaborative study.

613
614 In all fields of application, the extent to which
615 commutability has been established should be
616 clearly identified, as should any specific
617 limitations of use identified in the commutability
618 study.

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622 In line with developments in other fields of reference standard characterization, a
623 requirement to define what the biological reference standard measures is included in this
624 revised Guideline.

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In other fields this is referred to as definition of the "measurand". For biological reference standards the measurand may be a protein

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629 structure, a biological activity or an
630 immunological activity. In most cases, the
631 definition of the measurand will be reflected in
632 the procedures used to characterise and assign a
633 value to the standard. Thus standards intended
634 for calibration of bioassays will generally be
635 characterised using bioassay procedures, those
636 for immunoassays using immunoassay
637 procedures, and so on.

638
639 In a limited number of cases, and in particular in
640 cases where the material is sufficiently well
641 characterised to allow a complete physico-
642 chemical description, definition of the
643 measurand be achieved using a reference
644 method distinct from the routine assay
645 procedures. This approach is comparable to that
646 used in clinical chemistry, for analytes which,
647 whilst routinely assayed by immunoassays, may
648 be measured as defined molecular entities by
649 spectroscopic or other methods.

650 Examples include:

651 - the IS for somatropin (recombinant growth
652 hormone), used as a primary calibrator for
653 clinical immunoassays for growth hormone, is
654 assigned a value in mg, traceable to amino-acid
655 analysis of a physico-chemically defined
656 preparation.

657 - synthetic DNA standards, used in the
658 calibration of PCR assays, may be assigned a
659 value based on phosphate determinations of a
660 physico-chemically defined synthetic
661 polynucleotide.

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695 calibration of secondary preparations, whether of regional, national or more limited status;

696 these latter preparations are then used routinely.

697

698 **1.2 Definitions.**

699

700 Reference standards are materials which are used as calibrators in assays. WHO provides
701 reference standards for a range of substances which have been considered to be “biologicals”
702 (see below), and which includes, but is not restricted to proteins, antigens, vaccines, antisera,
703 blood products and nucleic acids. WHO reference standards are provided for the calibration
704 of assays based on interactions of components of living systems, including those based on
705 biological function, immunological reactivity, enzyme activation and enzyme amplification,
706 and serve as global, “highest order” measurement standards for the analytes they define.

707

708 The definition of a medicinal substance, used in treatment, prevention or diagnosis, as a
709 “biological” has been variously based on criteria related to its source, its amenability to
710 characterization by physico-chemical means alone, the requirement for biological assays, or
711 on arbitrary systems of classification applied by regulatory authorities. For the purposes of
712 WHO, including the present document, the list of substances considered biologicals is
713 derived from their earlier definition as “substances which cannot be fully characterized by
714 physico-chemical means alone, and which therefore require the use of some form of
715 bioassay”. However, developments in the utility and applicability of physico-chemical
716 analytical methods, improved control of biological and biotechnology-based production
717 methods, and an increased applicability of chemical synthesis to larger molecules, have made
718 it effectively impossible to base a definition of a biological on any single criterion related to
719 methods of analysis, source or method of production. Establishment of WHO measurement
720 standards for any substance or class of substances is therefore based on an evaluation of
721 current analytical methodologies, and where biological, immunological or enzymological
722 methods are employed, an evaluation of the need for global measurement standards for
723 calibration of these methods

724

For example, certain small proteins, such as
725 cytokines and hormones, classed as “well-
726 characterized”, are now considered to be
727 appropriately defined by physico-chemical

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728 methods. Nonetheless, the need for biological
729 measurement standards may be dictated by the
730 need to define the specific activity of new
731 products, or by the on-going requirement to
732 demonstrate specific activity of production
733 batches. In the diagnostics field, the requirement
734 for global measurement standards for otherwise
735 well characterised proteins and other
736 macromolecules is driven by the routine use of
737 comparative assay procedures such as
738 immunoassays and nucleic acid amplification
739 tests, and by the absence of reference methods for
740 the definition of the analyte in absolute terms in
741 reference materials.

742

743

744 The present document defines the major classes of WHO reference standard, and sets out
745 guidelines and criteria for their preparation, characterization and establishment. The
746 provisions of the document apply to each of the three classes of WHO reference standard
747 described below, except where specific modifications are described.

748

749 The principal class of WHO reference standard is the *international biological*
750 *measurement standard* (IS). These are substances, classed as “biological” according to the
751 criteria outlined above, which are provided to enable the results of biological assay or
752 immunological assay procedures to be expressed in the same way throughout the world. The
753 value assignment by the World Health Organization is in terms of an International Unit or
754 another suitable unit. Provided the candidate material has been shown to be suitable for its
755 purpose, the unitage is attributed to a first international standard in an arbitrary manner after
756 an international collaborative study has been completed. Activities in International Units are
757 assigned to replacement international standards, where appropriate, by comparing them with
758 the previous standard.

759

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760 An example of an IS that is assigned a unitage
761 other than an IU is thromboplastin. The 3rd IS
762 for thromboplastin, human, recombinant, plain is
763 assigned an International sensitivity index of
764 0.940.

765
766 *An reference reagent* is a WHO reference standard, the activity of which is
767 defined by the World Health Organization in terms of a unit. This category of
768 reference standard is intended to be interim and replacement reference reagents are
769 not envisaged. Sufficient information should have accrued in the period following
770 establishment to allow consideration of the reference reagent as an IS. Only when a
771 material established as a reference reagent is finally established as an IS will the
772 potency be expressed in IU. It is expected that the formally assigned potency, in IU,
773 following evaluation in an international collaborative study, be identical to the
774 assigned unitage. Assignment of a different value would only be done on basis of
775 sound scientific reasons. Specific requirements for the establishment of reference
776 reagents, as distinct from the general requirements applicable to WHO International
777 Biological Measurement Standards, are set out in Section 8.

778
779
780
781 The class of reference reagent was established in
782 response to the speed of development of some
783 new biological products (6). A need often
784 exists from both regulatory and scientific
785 considerations for reference standards with an
786 official status conferred by WHO to be
787 available before the clinical utility of such new
788 biological products becomes apparent. In such
789 cases, the full programme of establishment of
790 an IS may not be justified as the material may
791 have limited use until the clinical utility of the
792 biological product is established. In order to

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793 shorten the time between the preparation of a
794 candidate material and its distribution, it is
795 sufficient for a limited number of laboratories
796 to examine a characterised product and agree to
797 the assignment of potency as expressed in
798 units. As a minimum, the bulk material used in
799 the preparation shall have been shown to retain
800 biological activity consistent with the assigned
801 unitage by a competent laboratory, for example
802 the manufacturer, and this biological activity
803 shall have been confirmed by an independent
804 laboratory, preferably a WHO collaborating
805 laboratory. The candidate preparation should be
806 shown to meet the specifications for filling and
807 stability as defined in this document. The WHO
808 Collaborating Centre shall provide WHO with
809 the necessary information on the source and
810 characteristics of the preparation.
811 Physicochemical characterization should be
812 included if at all possible. It is not intended that
813 such reference reagents are product specific.
814 Such proposals may be submitted to WHO (see
815 section 7 for format of collaborative study
816 reports for reference standards).

817
818 *An international reference panel* is a group of reference materials established to
819 collectively aid evaluation of assays or diagnostic tests. International Reference Panels
820 comply in all respects with the general requirements for WHO reference standards set out
821 in this document, except that in some cases it may not be necessary to assign unitages to
822 each individual member of a panel.

823
824
825

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826

827 **1.3 Glossary**

828

829 In addition to the terms defined above, a number of terms are used through out this document
830 that merit further explanation. The meaning of these terms in the context of this document is
831 given in this glossary.

832

833 Baseline samples. Samples that are retained under optimal storage conditions to retain
834 biological or immunological activity and that are used for comparison purposes. The baseline
835 samples will need to be stored at a lower temperature than the storage temperature used for
836 the reference standard.

837

838 Biological tests (bioassay). A biological test is a procedure for the estimation of the nature
839 or potency of a material by means of the reaction that follows its application to some
840 elements of a living system (examples include animals , tissues, cells, receptors and
841 enzymes). The potency of the material being measured is often defined in International Units
842 or, in some circumstances, may be defined in SI terms, by comparison with the reaction of
843 the system to a biological reference preparation.

844

845 Continuity. The concept that measurements in terms of the IU defined by a replacement
846 reference standard are as similar as possible numerically to measurements in terms of the IU
847 defined by the previous reference standard. This ensures that measurements made in
848 biological and immunological tests can be compared over time.

849

850

851 Commutability. In general terms the concept of commutability seeks to establish the extent to
852 which the reference standard is suitable to serve as a standard for the variety of samples being
853 assayed. The way in which this is done may vary according to the intended application.
854 Details of options to assess commutability are given in the section on "General
855 Considerations".

856

857 Immunological tests. A procedure which requires the use of antigens and/or antibodies to
858 measure the analyte in a biological product or sample.

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859

860 International unit. The unitage assigned by the WHO to an International biological Standard.

861

862 In vitro diagnostic devices. Tests for the detection of diseases, such as blood borne pathogens
863 that can be transmitted via blood and blood products, or conditions such as pregnancy.

864

865 Secondary reference standards. Reference standards established by regional or national
866 authorities, or by other laboratories, that are calibrated against and traceable to the primary
867 WHO materials and are intended for use in routine tests.

868

869 Traceability. Property of the result of a measurement or the value of a standard whereby it
870 can be related to stated references, usually national or international standards, through an
871 unbroken chain of comparisons.

872

873 Validation. Confirmation, through the provision of objective evidence, that requirements for a
874 specific intended use or application have been fulfilled.

875

876 Uncertainty. An estimate attached to a test result which characterizes the range of values
877 within which the true value is asserted to lie.

878

879

880

881 **1.4 Nomenclature issues for biological reference standards**

882

883 During the course of the WHO programme on biological standardization, some
884 categories of reference standard have been established and later discontinued, for example
885 International Reference Preparations and International biological reference reagent.

886

887

For transparency and to avoid any confusion in
888 use or in the literature, reference standards that
889 were established in categories that are now
890 obsolete retain their designation and have not
891 been reclassified. However, when a preparation

888

889

890

891

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892 with an obsolete name is replaced, the new
893 nomenclature should be used. The pathway from
894 one class of name to the next should be clearly
895 explained in the memorandum that accompanies
896 the reference standard.

897
898 WHO standards for any given substance are identified by the assigned ordinate, as in
899 the 1st International standard for, the 2nd International Standard etc. It should be
900 recognised that great care must be taken in the use of this system of nomenclature to avoid
901 confusion with primary and secondary (eg working) standards. Where, as is usually the
902 case, establishment of a 2nd International Standard is accompanied by disestablishment of the
903 1st, it should be emphasized that the 2nd standard has effectively replaced the 1st as the highest
904 order reference standard, and critically, as the sole definition of the unit.

905
906 Although the source of some potential confusion about the hierarchy of standards, as
907 outlined above, this system of nomenclature has proved useful in situations where the
908 International Standard has been replaced on a regular basis, particularly in unambiguous
909 identification in the literature of which WHO standard published results are related to.

910
911 The year of establishment of a WHO standard should be given in the title of the
912 preparation.

913
914 During the course of the WHO biological standardization programme, a number of
915 examples have arisen where a native reference standard is replaced by a recombinant
916 material. In this case, the recombinant nature of the reference standard should be indicated in
917 the title of the preparation.

918
919 An example would be the 2nd IS for interferon B,
920 fibroblast which was replaced by the 3rd IS for
921 interferon B, human, recombinant, glycosylated
922 (11).

923

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924 When a native reference standard is replaced by a recombinant material a number of
925 factors should be considered.

926

927

It may be desirable to retain a sufficient stock of
the replaced native material so that any future
new or replacement recombinant standard may
be calibrated against the natural reference
standard (7, page 23). Consideration should also
be given to whether the native material should
be disestablished, or, where natural and
recombinant material may be regarded as
separate analytes, retained as a separate
standard. Follicle stimulating hormone is an
example of where the latter principle has been
applied.

928

929

930

931

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935

936

937

938

939

940 An international non-propriety name (INN) may be in existence for the material for
941 which a reference standard is established. Unless the reference standard is intended to be
942 used to standardise only that material complying with the definition of the INN, the INN is
943 not included in the title of the material, but is included in the memorandum sent out with the
944 material.

945

946

The point is illustrated by the example of Tissue
Plasminogen Activator (TPA). One preparation of
recombinant TPA has been assigned the INN
Alteplase. Whilst the International Standard for
TPA was prepared from Alteplase, it is intended for
use as a standard for TPA assays for TPA of all
sources, and therefore carries the title of the
International Standard for TPA, rather than the
International Standard for Alteplase.

947

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956 Where a reference standard is considered by the ECBS to be suitable for a restricted use
957 only, this should be included in the title of the preparation.

958

959 An example would be “the 1st IS for *a* , for
960 immunoassay”.

961

962 **1.5 Purpose of these Recommendations**

963

964 WHO designates certain centres as International Laboratories for Biological Standards
965 having the responsibilities of serving as custodians and distributors of international biological
966 reference standards. These laboratories have also been responsible for identifying needs for
967 such reference standards, obtaining the materials and preparing and studying the batches
968 either themselves or by other laboratories collaborating with them. The expansion of the
969 scope of work undertaken in biological standardization has led to a number of other
970 laboratories and organizations becoming involved in making preparations that may ultimately
971 be offered to WHO for consideration as biological standards. For this reason, the WHO
972 Expert Committee on Biological Standardization has recommended that all proposals by
973 international associations and other bodies for the establishment of international biological
974 reference standards, should be submitted to WHO so as to avoid duplication of effort (12).

975

976 Part A of these Recommendations is intended to reflect best established practice for
977 preparation, characterization and establishment of international biological reference
978 standards. It therefore serves as guidance for any laboratory or organization that becomes
979 involved in the preparation and testing of candidate materials intended for such a purpose.

980

981 Decisions on setting priorities in developing WHO International Standards or interim
982 reference standards should be based on criteria specified in Appendix 1.

983

984 **1.6 Safety considerations**

985

986 Many biological materials, including those of human origin, intended for the
987 preparation of an international biological reference standard must be considered as potentially
988 hazardous. For reasons of safety in handling and use, the material itself or its original matrix

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989 (e.g. blood) must be obtained appropriately and screened for the presence of infection or
990 other safety hazard. Blood should be obtained from donors who meet current international
991 requirements (13). Non-human animal proteins should meet current WHO requirements (18).

992

993

Screening will involve as a minimum the testing currently required for human blood and plasma, for example for the presence of hepatitis B surface antigen and markers for human immunodeficiency viruses (HIV), hepatitis C virus and for other relevant pathogens (13).

994

995

996

997

998

999

1000

If tests for the presence of infectious markers, (e.g. HIV markers) are not required for reference standards intended for the diagnosis of that infection (e.g. HIV infection), suitable evidence of proper inactivation should be provided. The geographic area from which the source material is obtained should be recorded.

1001

1002

1003

1004

1005

1006

1007

The actual or potential infectivity of biological materials of non-human origin, especially those derived from viruses or bacteria, should be taken into account. Suitable procedures may be applied to inactivate micro-organisms or their components and the effectiveness of this inactivation should be demonstrated.

1011

1012

The impact of any inactivation process on the fitness for purpose of the candidate standard should be investigated

1013

1014

1015

1016

Furthermore, it is essential that appropriate precautions are taken to ensure that shipments of biological reference preparations comply with international regulations on transport of infectious substances (14).

1019

1020

It is essential that suitable precautions are taken in the user laboratories in handling and disposal of biological materials to avoid possible infection. This is particularly important

1021

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1022 when the material is known or intended to be infectious. A safety data sheet (see Appendix 2)
1023 is provided with each reference standard.

1024

1025

1026 **2. Quality Assurance**

1027

1028 **2.1 Quality management system**

1029

1030 Biological reference standards should be obtained, processed, stored and dispatched
1031 under a defined quality management system. International recommendations are available
1032 from ISO (15,16,17). It is desirable that the quality management system is assessed as
1033 satisfactory by an independent body.

1034

1035 Other essential components of the process of standards development may be partly, or
1036 entirely outside the control of the organizing or coordinating laboratory. These include:

- 1037 - preparation and characterization of candidate materials in donor laboratories
- 1038 - characterization of candidate materials and trial formulations in testing
1039 laboratories
- 1040 - contribution of WHO and other consultative committees to study design
- 1041 - performance of testing by participants in collaborative studies
- 1042 - review of data and formal establishment by ECBS.

1043

1044 Although such activities may fall outside the possible scope of a formal quality system, it is
1045 strongly recommended that processes and documentation compliant with recognised quality
1046 standards, are, as far as possible implemented and followed.

1047

1048 Managing organizations are encouraged to
1049 review continuously the entire process of
1050 standards development, from the initial
1051 sourcing of material through to the
1052 laboratories participating in the
1053 collaborative studies, with a view to
1054 bringing essential and controllable aspects

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1055 of the process within defined quality
1056 management systems.

1057

1058

1059 **2.2 Records**

1060

1061 It is essential that complete records are kept, in compliance with quality system
1062 requirements, relating to, inter alia,

1063

- 1064 • The background and proposals for preparation of the intended reference standard
- 1065 • The responsible persons and their defined roles
- 1066 • Certificates of analysis of bulk materials intended for use as an international reference
1067 standard. If this is plasma or serum based, such information as is available about the
1068 donors,

1069

1070 Information about donors may include
1071 details of the donation centre, the gender
1072 and age of donors, and records of ethical
1073 approval for the donations.

1074

- 1075 • the procedures and tests which have been performed before, during and after filling into
1076 containers, including quality control tests for residual moisture and homogeneity
- 1077 • stability studies,
- 1078 • raw data from collaborative studies,
- 1079 • reports and recommendations
- 1080 • records of agreement or otherwise of participants
- 1081 • storage, inventory and dispatch of the reference standard.

1082 Such records form the basis of the International Unit as the fundamental unit of measurement
1083 for any given analyte. They should therefore be retained even after a standard is replaced,
1084 and should be kept until such time as the International standard, and hence the International
1085 Unit, is discontinued and not replaced.

1086

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1087 2.3 Validation of methods

1088 The quality system should clearly identify critical equipment and technology and set out
1089 procedures for validating and maintaining its functionality. Such critical equipment includes,
1090 but is not restricted to:

1091

1092 - analytical equipment for determination of freezing and other transition points

1093 - liquid handling equipment for dispensing into ampoules/vials

1094 - freeze-driers

1095 - isolators for sterile fills

1096 - ampoule/vial sealing equipment

1097 - equipment for carrying in-process controls

1098 - air-filtration equipment for maintenance of sterile/clean rooms

1099 - sterilization, washing and water purification equipment

1100 - storage equipment.

1101

1102

1103 **3. Assessment of need and procurement of materials**

1104

1105 **3.1 Assessment of need**

1106

1107 International biological reference standards may be needed for:

1108 • the assay or characterization of a biological product approved, or intended for approval,
1109 for use in medical practice and distributed in more than one country,

1110 • the identification of a biological material of importance in medical or laboratory practice,

1111 • the calibration of regional, national or laboratory biological reference standards.

1112

1113 The WHO Expert Committee on Biological Standardization will not normally establish
1114 biological reference standards intended solely for research purposes.

1115

1116 Exceptions are made where the availability of

1117 biological reference standards for research

1118 purposes may be of international public health

1119 significance. An example would be the WHO

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1120 Reference Reagent for human brain, variant
1121 CJD, established to facilitate research to develop
1122 assays to detect the agent of vCJD (11a).

1123

1124 When a need for an international biological reference standard is identified by another
1125 organization, it is essential that WHO should be informed of this and of whether that
1126 organization intends to proceed with preparation of the material, so as to avoid unnecessary
1127 duplication of effort. Co-ordination with other standards setting bodies is important in this
1128 respect.

1129

1130 A decision tree (Appendix 1) aids allocation of priority to requests for new and replacement
1131 biological reference standards.

1132

1133 **3.2 Nature, source and storage of bulk material**

1134

1135 A fundamental tenet of biological standardization is that the behaviour of the reference
1136 standard should resemble as closely as possible the behaviour of test samples in the assay
1137 systems used to test them. Choice of candidate materials should reflect this principle of assaying
1138 “like against like”.

1139 . The bulk material selected should have a high degree of stability and a specific activity or
1140 concentration sufficient for the purposes of the assays or tests for which it is to be used.

1141 Although the material does not necessarily have to be of the highest purity, no other
1142 substances present should interfere with the procedures in which the material is to be
1143 employed.

1144

1145 Generally speaking, the nature of the candidate
1146 material will reflect the current “state of the art”
1147 for any given analyte. Thus a therapeutic protein
1148 will generally be essentially pure, and will be
1149 provided with a certificate of release describing
1150 its specific biological activity, its physico-
1151 chemical characterisation, and its freedom from
1152 significant contaminants. Plasma products will

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1153 be representative of current manufacturing
1154 capability, and in addition will be provided with
1155 certificates demonstrating compliance with
1156 current safety and ethical requirements. Vaccine
1157 preparations will represent current practice in
1158 preparation of microbial immunogens. Where
1159 the nature of the reference standard does not
1160 permit such detailed characterization (eg plasma
1161 antibodies) then the characterization of the bulk
1162 material should as a minimum describe the
1163 biological activity in relation to the activity
1164 intended to be standardised.

1165

1166 The bulk material will usually be obtained from a single source. It may consist of part or all
1167 of a single batch. This may be difficult to achieve for standards derived from human plasma,
1168 in which case a small number of large samples are the preferred source material, rather than a
1169 large number of small samples.

1170

1171 For bulk materials manufactured by an industrial
1172 process, a certificate of analysis of the batch(es)
1173 should be provided by the donor of the bulk
1174 material. This information will not be disclosed
1175 to users without permission from the donor.

1176

1177 If it is necessary to prepare the bulk by pooling material from more than one batch or
1178 source, the procedure employed should ensure that the pooled material is mixed thoroughly
1179 and is homogeneous. For bulk liquids containing proteins, care should be taken to avoid
1180 denaturation during mixing. In addition to any studies that may have been made on the
1181 individual batches before pooling, the suitability of the homogeneous blend should be
1182 demonstrated.

1183

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1217 and have appropriate physical properties. Liquid bulk materials should usually be stored
1218 frozen and special precautions should be taken to achieve proper freezing. Liquid bulk
1219 materials may be stored at 2-8°C provided that they are sterile or contain an antimicrobial
1220 preservative. In all cases, the containers of bulk material should be able to withstand the
1221 conditions of freezing, storage, thawing, opening and, if applicable, freeze-drying. In all
1222 cases, the storage conditions should ensure that the biological activity of the material is
1223 conserved.

1224

1225 **4. Distribution into final containers**

1226

1227 **4.1 General considerations**

1228

1229 An important requirement to be met by a batch of an international biological reference
1230 standard is that the material in every final container in the batch should be within specified
1231 limits, as defined below, in terms of composition, quantity, potency and stability.

1232

1233 In order that all the samples of a preparation are homogeneous, they should all be
1234 derived from the same homogeneous bulk, and should all be processed together in one
1235 working session. Processing should be performed in an environment with an appropriate low
1236 bioburden level. The bulk material is distributed, usually in liquid form to achieve high
1237 precision of fill, into a number of suitable containers. The contents of the containers are dried
1238 from the frozen state. This process may also be applied to insoluble solids that can be
1239 suspended in a suitable liquid. Materials that cannot be dried satisfactorily may, after
1240 dispensing, be stored as liquids provided that stability is retained under the storage conditions
1241 employed.

1242

1243 Suitable safety precautions should be taken to protect personnel and the environment
1244 from exposure to any potentially infectious or harmful material.

1245

1246 **4.2 Treatment of liquid bulk materials**

1247

1248 The choice of process and the extent of processing required for preparing the final bulk
1249 for filling will depend on whether the liquid bulk is a true solution, a colloid, or a suspension.

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1250 In all cases the processing should ensure that the product is homogeneous during filling, and
1251 measures should be taken at all stages to avoid contamination of the material. Liquids may
1252 have to be treated chemically or physically to control microbial contamination or to remove
1253 particles or aggregates of active material. Water-soluble materials are dissolved at a suitable
1254 concentration in diluents, buffers or stabilizing solutions.

1255

1256 These solutions should be prepared from water
1257 of a purity comparable to double glass distilled
1258 water, or higher, and pyrogen-free where
1259 appropriate

1260

1261 If inclusion of an antimicrobial preservative is necessary, it should be one that will not
1262 affect the intended use of the preparation or volatilize during the drying process, and not
1263 decrease the stability of the preparation.

1264

1265 The choice of preservative is an important
1266 consideration since some countries place
1267 restrictions on acceptable preservatives. The
1268 choice of preservative should be justified and
1269 records of this retained.

1270

1271 Cresol, phenol or sodium azide (which may
1272 form explosive compounds with metals) should
1273 not be used as preservatives in a preparation that
1274 is to be freeze-dried. .

1275

1276 A biologically active substance is frequently present in a container of the reference
1277 standard in such small amounts that a bulking agent has to be present in the solution for
1278 filling to allow a visible freeze-dried plug of suitable size to be formed. In some instances,
1279 added materials are chosen to prevent or limit adsorption of the active substance on to the
1280 internal glass wall of the container and structural changes affecting biological activity that
1281 may occur during freeze-drying. Any added substance should not have adverse effects on the

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1282 activity of the material nor interfere with the assay or test for which the preparation is
1283 intended.

1284

1285 If a protein carrier, such as human albumin is
1286 used, it should comply with current
1287 requirements for blood products for freedom
1288 from contamination (13, 18) and proteolytic
1289 enzymes should be minimal. Certain sugars,
1290 particularly those with reducing groups (e.g.
1291 lactose) should be avoided as bulking agents as
1292 they can form stable complexes with amino
1293 groups in proteins.

1294

1295 Preliminary freeze-drying trials with extensive analysis of the dried material may be
1296 necessary to establish that an added substance has not affected the desired characteristics and
1297 potency of the active material. Such studies may include investigating the stability of the
1298 reconstituted trial preparation.

1299

1300 It is normal practice for the contents of each container to be sufficient for several
1301 analyses or assays. However, after reconstitution of a lyophilized material, it may be
1302 desirable to subdivide the resulting solution into several containers, each sufficient only for
1303 one or two assays. These containers must be stored in such a way that their contents remain
1304 unchanged. For scarce materials, the amount chosen to be filled into each container should
1305 take into account the need to conserve the material.

1306

1307 **4.3 Treatment of solid bulk materials**

1308

1309 It is recommended that filling solid bulk materials is avoided. However, materials that
1310 are insoluble in water or less stable in a freeze-dried form may have to be distributed into
1311 containers as powders. In such a case, special precautions should be taken to ensure that both
1312 the bulk material and the samples taken from it are homogeneous. Special mixing and
1313 sampling devices may be necessary.

1314

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1315

1316

1317 **4.4 Quality of final containers**

1318

1319 Heat-sealed ampoules are used in preference to stoppered vials for international
1320 reference standards. A sealed glass ampoule does not allow exchange of gases and moisture
1321 with the atmosphere and the long-term stability of biological materials is generally much
1322 greater under these conditions.

1323

1324 Stoppered vials may be used for certain types of biological material, such as infectious
1325 preparations. Rubber or elastomer stoppered vials also may be considered for the preparation
1326 of international reference standards that are used for qualitative purposes.

1327

1328 Where possible, a small number of sealed glass
1329 ampoules of the material should also be
1330 prepared so that a baseline is available for
1331 checking stability should the need arise.

1332

1333 Containers should be of neutral (borosilicate) glass type I of appropriate quality, for
1334 example complying with the current requirements of the European Pharmacopoeia or the US
1335 Pharmacopoeia. The glass must be free from stresses and the containers must be able to
1336 withstand sterilization by heat and temperature stresses, such as those resulting from rapid
1337 freezing to -80°C. Actinic (brown) glass may be necessary for photosensitive materials but
1338 does not allow the contents to be seen clearly. If stoppered vials are used, the closures should
1339 be of appropriate quality, for example complying with current pharmacopoeial requirements
1340 for closures for injections.

1341

1342 Containers and closures should not affect the
1343 stability of biological standards and this may be
1344 shown through validation studies.

1345

1346 The volume of the containers used depends on the amount of material required in each
1347 but a capacity of about 5 ml is generally suitable for fills up to 1 ml in volume.

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1348

1349 A specification for purchase of containers, and if necessary closures, should be
1350 established. Batches intended for use should be shown to conform to the specification. The
1351 shape and size of ampoules should be such that they can be filled easily, sealed by fusion of
1352 the glass without adverse effects on the contents, opened easily and their contents removed
1353 without difficulty.

1354

1355 It is advisable to use flat-bottomed ampoules for
1356 preparations to be lyophilized since this ensures
1357 good thermal conductivity between the bottom
1358 of the ampoule and the top of the shelf in the
1359 freeze-drier.

1360

1361 The containers should be cleaned by a process that does not involve use of a detergent.
1362 If the clean containers are to be stored at any time before filling, they should be placed in
1363 sealed dust-proof containers.

1364

1365 An example of cleaning without detergents is by
1366 heating in distilled water in an autoclave, by
1367 steaming in hydrochloric acid (20 g/l), or by
1368 acetic acid (2% v/v), or by ultrasonic treatment.
1369 The containers should then be rinsed several
1370 times with clean water and finally with distilled
1371 water. Steam admitted to autoclaves for
1372 cleaning or sterilization of glassware must be
1373 free from any volatile or non-volatile
1374 compounds that may be present as a result of the
1375 use of boiler-water additives. If steaming in
1376 hydrochloric acid is carried out in an autoclave,
1377 great care must be taken to remove residual
1378 traces of the acid from the autoclave afterwards.
1379 The washed containers should then be sterilized

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1380 by dry heat in a clean, grease-free and silicone-
1381 free oven. .

1382

1383 **4.5 Distribution into containers**

1384

1385 *4.5.1 General considerations*

1386

1387 Containers are usually filled before labelling.

1388

1389 Each container in the batch either should be
1390 permanently marked with some form of in-
1391 process identification of the material being filled
1392 or a quality system should be in place to assure
1393 the separation of containers from different
1394 batches.

1395

1396 If containers are marked, the form of marking
1397 should not scratch the surface of the glass.

1398

1399 Containers should be filled from a single homogeneous bulk material. A liquid bulk
1400 should be stirred continuously during filling and held at constant temperature in order to
1401 ensure that homogeneity is maintained throughout the filling process. Exposure to direct
1402 sunlight should be avoided.

1403

1404 Filling should be carried out in a clean environment, for example a clean room or in a
1405 laminar-flow cabinet equipped with a HEPA filter in order to avoid any form of
1406 contamination.

1407

1408 Criteria for the quality of the air, or for the
1409 performance of air filtering systems should be
1410 written into the quality system, and relevant
1411 parameters monitored accordingly.

1412

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1413 ...

1414

1415 A sample for testing cannot be assayed more accurately than the reference standard
1416 against which it is compared. Since a reference standard in the dried state has to be
1417 reconstituted, introducing further variability, the precision of fill should be as high as possible
1418 and the coefficient of variation as low as possible in order to minimise inaccuracy of assay.
1419 Assays of biological materials often differ considerably in their reproducibility. In setting a
1420 target precision of fill (maximum coefficient of variation) for a biological reference standard
1421 for quantitative measurement, regard should be paid to the reproducibility inherent in the
1422 assay procedure(s) in which it will be used.

1423

1424 There is no formal pass/fail criteria for the
1425 production quality control parameters given
1426 below. The important criterion is fitness for
1427 purpose. Nevertheless the criteria specified
1428 below are expectations that are fulfilled by the
1429 vast majority of WHO biological reference
1430 standards.

1431

1432 *4.5.2 Liquid fills*

1433

1434 For each filling run, about 1-2% of the containers should be selected and weighed
1435 before and after filling in order to check the variation in the amount (volume or mass) filled
1436 into each container. The precision of fill or coefficient of variation (standard deviation
1437 divided by the mean) can be derived from the data obtained. The sample should be assessed
1438 for any consistent significant change in filling weights over the course of the process. The
1439 selection of the containers should be according to a procedure designed by a biometrician to
1440 ensure as far as possible that the sample is representative of the filling run.

1441

1442 The nature of a liquid influences the precision with which it can be dispensed for
1443 filling. A coefficient of variation not greater than 0.0025, that is 0.25%, is achievable for
1444 aqueous solutions with a 1ml fill volume. However, more viscous liquids cannot usually be
1445 dispensed with this degree of precision. For liquids such as plasma or cellular materials, a

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1446 coefficient of variation on a 1ml fill of < 1% is realistic. In the case where a reference
1447 standard is not to be freeze-dried, the volume filled into the container should be a slight
1448 excess of the volume intended to be extracted by the user.

1449

1450

1451 *4.5.3 Powder fills*

1452

1453 It is recommended that powder fills are avoided. Powder fills have been used formerly
1454 when the amount of material is not a limiting factor. They may be necessary for water-
1455 insoluble materials.

1456

1457 Most powders can be fed into containers by means of an automatic filler, but spoons of
1458 suitable size may also be used. Large variations in the amount per container may be
1459 unavoidable although this may be unimportant if an exact quantity of the contents is weighed
1460 at the time of use. Special precautions will be necessary for solids that are hygroscopic or
1461 efflorescent as well as for those that may acquire an electrostatic charge and stick to the
1462 inside of the container.

1463

1464 **5. Processing of filled containers**

1465

1466 **5.1 General considerations**

1467

1468 International standards should be prepared using conditions in which it has been
1469 demonstrated that the biological activity and other significant properties of the material are
1470 not degraded or lost, that the activity of the final preparation is stable, and that the biological,
1471 physical and chemical properties of the standard are compatible with its intended use. Where
1472 the standard is a replacement, much of this information will be available. However, new
1473 standards will require Research and Development to determine suitable conditions and
1474 formulations. This is achieved by carrying out and analysing small scale trial fills, using
1475 conditions which mimic as closely as possible the conditions in the large scale definitive fill.
1476 The program of research and development should be clearly identified and recorded. The
1477 records should also specify details of baseline samples that are retained for comparison

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1478 purposes, which should included both non-freeze-dried samples stored at -150C (frozen
1479 baselines), and also freeze-dried samples stored at -150C.

1480

1481

1482 The processing of filled containers should be completed under optimal conditions. It is
1483 essential to ensure that all the containers in a batch are processed together from the time of
1484 filling until completion of the process so that they are subjected to the same conditions at the
1485 same time. Only one material should be processed at a time in the freeze-drier since cross-
1486 contamination has been demonstrated when more than one material is present.

1487

1488 Ampoules should only be sealed by fusion of the glass. If stoppered vials are used, it
1489 should be borne in mind that rubber or elastomer closures may be unsatisfactory in long-term
1490 storage since their physical properties may change and they may allow exchange of gases
1491 with the surroundings.

1492

1493 Samples should be taken at appropriate times during processing so that the baseline
1494 properties and potency of the material may be assessed. The samples, suitably sealed, should
1495 be preserved in the vapour phase of liquid nitrogen. They can be used to evaluate the effects
1496 of processing on the biological material and to confirm, for example, that there has been no
1497 change in composition or loss of biological activity.

1498

1499

1500

1501

1502 **5.2 Processing of materials that are to be freeze-dried**

1503

1504 *5.2.1 Freezing*

1505

1506 The freezing process is very complex. When liquid containing water is frozen, pure ice
1507 forms first and the dissolved components become progressively concentrated in the
1508 remaining solution. Electrolytes usually crystallise but biological materials such as proteins
1509 and carbohydrates usually do not. Instead the viscosity of the solution increases to the point
1510 where it can be considered to be a glass and the whole liquid has become solid, i.e.

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1511 completely frozen. The liquid in the containers should be frozen to a sufficiently low
1512 temperature to ensure that this condition is reached.

1513

1514 This requires a temperature between about -
1515 20°C for sodium chloride solution to about -
1516 50°C for serum, but sometimes a liquid does not
1517 begin to freeze until well below its apparent
1518 “freezing temperature”, a phenomenon known as
1519 “supercooling”. The temperature at which any
1520 given solution is completely frozen should be
1521 determined in a preliminary study by a
1522 technique such as differential thermal analysis.
1523 Measurement of changes in electrical resistivity
1524 is less sensitive.

1525

1526 Depending on the rate of cooling and the temperature reached, the greatly increased salt
1527 concentration and pH changes in buffers may damage proteins and result in loss of their
1528 biological activity. Some antibodies, clotting factors and enzymes are known to denature
1529 under these conditions. Thus, the rate and temperature at which freezing is carried out are
1530 important in preserving the activity and solubility of the material, and the most suitable
1531 conditions should be determined experimentally. Sometimes, the precise conditions for
1532 successful freeze-drying of a given liquid can only be deduced from experience with similar
1533 freeze-drying operations.

1534

1535 5.2.2 Freeze-drying

1536

1537 The filled containers are usually processed in a shelf freeze-drier. The containers are
1538 arranged, usually on trays from which the base can be withdrawn, on temperature-controlled
1539 shelves in an evacuated chamber. The temperature of the material in the containers should be
1540 recorded continuously. If heat is applied to the shelves during the process, care should be
1541 taken to ensure that it is applied uniformly. Water vapour sublimates from the ice in the frozen
1542 liquid and forms as ice on a condenser at a lower temperature than that of the shelves.
1543 Sublimation of water draws heat from the material in the containers which is replaced by heat

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1577 containing a vacuum can be tested with a high
1578 frequency coil. All defective ampoules should
1579 be discarded. A suitable validation procedure
1580 may replace the need to test individual
1581 ampoules.

1582
1583 Vials may be sealed with rubber or elastomer
1584 caps held in place usually with an aluminium
1585 cover. On occasions, screw capped vials may be
1586 used.

1587
1588 The sealed containers should be labelled, stored at an appropriate temperature, and
1589 protected from light. Storage temperature is usually -20°C but may be lower.

1590

1591 **5.3 Procedure where freeze-drying is not used**

1592

1593 When liquid or solid preparations are not to be freeze-dried, the containers holding
1594 them may be filled with an appropriate gas before sealing.

1595

1596 This may be achieved by placing the filled
1597 containers in a chamber that is evacuated and
1598 filled with the pure, dry inert gas. This process
1599 should be repeated several times to remove
1600 residual air and moisture. The containers are
1601 then sealed.

1602

1603 **5.4 Labelling**

1604

1605

1606

1607 Each container must be marked with an identifying code unique to the batch which
1608 permits positive identification throughout the filling process. Materials intended to serve as
1609 international biological reference standards must not be labelled as such until they have been

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1610 formally established by the WHO Expert Committee on Biological Standardization. Once
1611 this is done, each container in the batch should be labelled to show the following items of
1612 information:

1613

- 1614 • The name “World Health Organization”.
- 1615 • The name and status of the preparation in the form “ International Standard (or Reference
1616 Reagent) for ...”.
- 1617 • The year in which the reference standard was established by the WHO Expert Committee
1618 on Biological Standardization.
- 1619 • The unique code allocated by the filling laboratory to enable the batch to be identified.
- 1620 • The storage conditions recommended for the material
- 1621 • A statement that the material is not for use in humans.

1622

1623 If the size of the label permits the following information may also be shown. If the size
1624 of the label is not sufficient, this information must be shown in the Instructions for Use that
1625 accompanies the standard:

1626

- 1627 • The potency or other parameter assigned to the reference standard. This is usually the
1628 number of International Units per container but may be the mass of solid containing one
1629 International Unit; or the number of International Units per milligram.
- 1630 • The name and address of the organization designated to hold and distribute the material.
- 1631 • A statement that the material should be used as directed in the Instructions for Use
1632 (package insert, safety data sheet) accompanying the reference standard.

1633

1634 **5.5 Characterization of the final product in the container**

1635

1636 The residual moisture content and residual oxygen content of the final product in the
1637 container should be determined and evidence of freedom from microbial contamination
1638 obtained. The final product in the container should be tested and found satisfactory for
1639 potency or biological activity, as appropriate.

1640

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1674

1675

The number of containers to be tested depends a

1676

on considerations of the test methods to be used,

1677

and the lot size of the batch; the number is

1678

determined by a pre-defined sampling plan. This

1679

is determined using at least three containers in

1680

order to confirm that the atmosphere within the

1681

container is inert and that the material is

1682

protected against oxidative change. Oxygen

1683

levels below 45 $\mu\text{mol/l}$ when determined at

1684

atmospheric pressure using for example an

1685

oxygen fuel cell meter or mass spectrometer

1686

have been shown to ensure adequate long-term

1687

stability.

1688

1689

Residual oxygen determinations may not be

1690

needed on every new batch of ampoules if the

1691

process is adequately validated.

1692

1693 *5.5.3 Characteristics and potency or biological activity*

1694

1695

It is essential that the biological material in the container is demonstrated to have

1696

retained its integrity, composition and potency, or biological activity, using appropriate

1697

methods.

1698

1699

1700 **5.6 Stability of the final product**

1701

1702

1703

Determination of the stability of reference standards, i.e., establishing the rate of loss of potency or activity, under a variety of conditions is desirable for three reasons:

1704

1705

1. To provide an estimate of the length of time for which the reference standard will remain suitable for its intended purpose under its defined storage conditions.

1706

1707

2. To define appropriate conditions for distribution of the reference standard to users.

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1708 3. To determine the extent to which the reference standard will retain its activity over
1709 time after reconstitution.

1710

1711 In most cases, no independent scale of measurement is available for the reference standard
1712 which itself serves to define its unit of activity, and hence no direct method of estimating the
1713 rate of loss of potency of the reference standard under its defined storage conditions is
1714 possible. Indirect and approximate methods are used for determining the rate of loss. These
1715 methods are generally based on the relationship between reaction rates and temperature given
1716 by the Arrhenius equation and a first order reaction rate is frequently assumed. Use of these
1717 methods requires that samples of the reference standard are stored at a range of elevated
1718 temperatures and tested for potency relative to the reference standard stored at lower
1719 temperatures.

1720

1721 Kirkwood (21) has described an iterative procedure based on a maximum
1722 likelihood approach for estimation of the parameters of the equation relating
1723 degradation rate to temperature.

1724

1725 Many biological products appear to exhibit Arrhenius type behaviour over a
1726 modest range of temperatures. However, as this relationship is approximate,
1727 particularly over wide temperature ranges, caution must be exercised in accepting
1728 the predicted rates of reaction. Reference standards are designed to be stable
1729 under defined storage conditions, and may also show no apparent loss of potency
1730 after storage at elevated temperatures. Experience has shown that reconstitution
1731 may be difficult after high temperature storage for some reference standards.
1732 Factors such as this must be taken into account when designing degradation
1733 studies. Lack of detectable degradation, and consequent lack of predicted
1734 stability, does not preclude the establishment of an IS.

1735

1736 An example of an International Standard where data appeared to follow the
1737 Arrhenius equation is the International Standard for thrombin, which gave a
1738 predicted loss of activity at -20°C of less than 0.1% per year (25).

1739

1740 Data from the thermally accelerated degradation study may also be used to
1741 predict likely loss of activity at higher temperatures which may occur during
1742 distribution of the reference standard, and these data may be used to define
1743 appropriate conditions for distribution.

1744

1745 The selection of suitable analytical methods for monitoring the stability depends on the
1746 nature and intended use of the substance. The number of laboratories involved in stability
1747 studies is generally fewer than the number involved in the main collaborative study to
1748 assess the suitability of the candidate material

1749

1750 Expiry dates are not assigned to biological reference standards, providing that long-term
1751 stability is predicted on the basis of existing data. In some circumstances further study, or
1752 monitoring on a case-by-case basis, taking into account data obtained from thermally
1753 accelerated degradation study, may be recommended by the ECBS. If there is a change in
1754 storage conditions of the reference standard at the custodian laboratory, new stability studies
1755 are required. Some samples of the reference standard should be stored at temperatures lower

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1756 than the customary storage temperature when the standard is initially prepared, to provide a
1757 low temperature baseline for long term stability studies.

1758

1759

1760

1761

1762

1763

1764

1765

1766

1767

For example, in an international collaborative study of the IS for thyroid stimulating hormone for immunoassay (26) samples were held at the storage temperature of -20°C and baseline samples held at -150 °C for 7,371 days (20 years); no difference was measured in the stability of the samples held at the two temperatures. The loss of activity for the preparation, coded 81/565, stored continuously at -20 °C was 0.04% per year.

1768

1769

1770

1771

Available information about the stability of the material after reconstitution should be given to users. Other information on factors that may affect properties of the reconstituted material, e.g. adsorption to particular types of container, should also be given.

1772

1773

1774

This type of information will be limited since the conditions of reconstitution and storage cannot be extensively studied within many collaborative studies.

1775

1776

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1794 **6. International collaborative studies**

1795

1796

1797

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1799

An international collaborative study must be carried out before any candidate biological reference standard can be considered for establishment by the WHO Expert Committee on Biological Standardization. The amount of work and resources required to carry out such a study should not be underestimated. For standardization projects carried out by WHO

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1800 standardization laboratories or other WHO collaborating centres , WHO, through the Expert
1801 Committee on Biological Standardization, should be informed of the intention of the
1802 collaborating laboratory to undertake the work and have given agreement in principle to
1803 consider establishment of the candidate material, in order to avoid unnecessary or duplicated
1804 work. In agreeing in principle to the undertaking of work leading to the establishment of an
1805 international Standard, WHO may, either through ECBS or through the activities of Working
1806 Groups with vested responsibility for specific topics, make recommendation on the broad
1807 outline of studies to be pursued. ECBS will not normally contribute to the specific detail of
1808 collaborative study design.

1809

1810 In some circumstances, WHO may establish collaborative links with other standardization
1811 organizations jointly to pursue specific standardization projects which have been prioritised
1812 and initiated independently. It is nonetheless desirable that through ECBS, WHO prioritises
1813 and endorses such projects before completion and establishment of the standard.

1814

1815 Collaborative studies should be organized by one or more scientist(s) familiar with the
1816 appropriate biological field, working closely with an experienced biometrician, and according
1817 to the general principles set out below.

1818

1819 **6.1 Aims of a collaborative study**

1820

1821 The purpose of a collaborative study is to demonstrate that the candidate international
1822 biological reference standard is suitable for its intended use. A list of potential aims of the
1823 study are given below but not all of these aims can be studied in a single study:

1824

- 1825 • Confirmation that the biological material has the properties and activity expected of it.
- 1826 • Demonstration that the candidate reference standard is suitable for calibration of other
1827 reference standards or examination of preparations from a variety of manufacturers or
1828 sources.
- 1829 • For reference standards intended for use in the diagnostics field, an assessment of
1830 commutability to clinical samples, where appropriate and feasible, should be considered
- 1831 • Comparison of two or more candidate materials.
- 1832 • Assignment of a potency or other parameter to the contents of the containers.

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- 1833 • Whether different assay methods (e.g., bioassays and immunoassays, in vivo and in vitro
- 1834 assays) measure the same or different properties of a proposed reference standard. This
- 1835 may include assessment of the effects of biologically active contaminants.
- 1836 • Comparison of a replacement batch against the current reference standard.
- 1837 • Provision of a reference standard for a substance for which validated assay methods are
- 1838 not available.

1839 An example is the WHO human CJD reference
1840 panel that was established in 2003 and is
1841 intended for assay validation (11a).

1842

- 1843 • Assessment of the stability of the proposed reference standard.
- 1844 • Assessment of the molecular integrity and composition of the reference standard.

1845

1846 The aims of the study should be defined at the outset, if appropriate in consultation with
1847 WHO and potential participants.

1848

1849 An international collaborative study of a
1850 candidate biological reference standard is a
1851 scientific study designed to provide soundly
1852 based advice for the ECBS on the characteristics
1853 of a proposed standard and its likely suitability
1854 for intended use. Collaborative studies provide
1855 valuable scientific information about the
1856 materials studied and the assay systems in
1857 current use which could not be obtained by any
1858 one individual laboratory.

1859

1860 **6.2 Planning and design**

1861

1862 An international collaborative study for characterization of a biological standard should
1863 be based on the principles of biological assay, designed according to sound statistical
1864 principles, and analysed and interpreted following sound statistical and biological principles.

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1865 Although there is no generic collaborative study design, the principles set out below should
1866 be followed.

1867 The details of the proposed collaborative study, and the underlying scientific rationale should,
1868 in all cases, be recorded, and these records retained throughout the time the standard is in use.

1869

1870 Each study is unique and requires current
1871 scientific knowledge about the structure and
1872 function of the biological material, the nature of
1873 assays currently available, the availability of
1874 potential study materials and the availability of
1875 potential participants. This requires both a
1876 biological scientist and a biometrician, ideally
1877 with experience of such studies, to bring
1878 together experience of the biological material
1879 and the bioassays for it.

1880

1881 The rationale for the proposed study design and
1882 the proposed statistical methods for analysis of
1883 the study should be outlined. Both study design
1884 and methods of analysis may necessarily change
1885 to reflect the data which the participants are able
1886 to submit.

1887

1888 A key decision that will influence the study design is the choice of unit (IU or SI)
1889 intended to be assigned to the candidate reference standard. The choice of unit, and rationale
1890 for the choice, should be explicitly stated in the study protocol. If the study is of a
1891 replacement reference standard the way in which continuity of the IU will be addressed is the
1892 key consideration in the study design that should be explicitly stated in the study protocol.
1893 The aim of continuity is that the IUs defined by a replacement reference standard are as
1894 similar as possible numerically to measurements in terms of the IUs defined by the previous
1895 reference standard. This is to ensure that measurements made in biological and
1896 immunological tests can be compared over time.

1897

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1898 It is necessary to decide which samples will be examined in the study. For example,
1899 test materials other than the candidate reference standard(s) may have to be obtained.
1900 Inclusion of too many samples should be avoided.

1901

1902 As an example, normal plasma pools may be
1903 included in studies of candidate reference
1904 standards for blood coagulation factors as a
1905 cross-check for the continuity of the IU. Where
1906 this is done, the study report should provide
1907 details of the normal donor pools used to obtain
1908 the normal plasma pool.

1909

1910 The study should be designed so that each assay generates internal evidence allowing
1911 assessment of statistical validity (for example, evidence of linearity and parallelism for
1912 parallel-line assays) and precision (27).

1913

1914 The number of participants will depend on the nature of the study, taking account of its
1915 aims, the number and type of assay systems included, the materials to be studied, the number
1916 of possible participants and their resources, and the capacity of the various assay systems.

1917

1918 Where appropriate, working groups may be
1919 formed to facilitate the development of
1920 standards. Guidance may be provided on the
1921 methods to be used and the selection of
1922 laboratories.

1923

1924 If the study is complex in design or new test procedures are being used, it may be necessary
1925 to include more participants than would be required, for example, for a study of a
1926 replacement standard using a well-defined pharmacopoeial assay method. If a new
1927 international biological reference standard is to be established with a defined unit of activity,
1928 a method for measuring the desired activity should exist already. If several assay methods are
1929 available, the material chosen should be suitable for use with as many of them as possible.

1930 The majority of studies are likely to include between five and 25 participants

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1931

1932

An example of an international collaborative

1933

study conducted according to the principles

1934

outlined above, was the study to establish the IS

1935

for low molecular weight heparin (2003) (add

1936

ref).

1937

1938

1939

Participants may be asked to carry out a specified minimum number of independent

1940

assays, or, if the assay procedure is known to be imprecise, a number sufficient to provide a

1941

mean estimate of acceptable precision. Duplicate assays may be requested. An independent

1942

assay is defined as one made using fresh dilutions from a newly opened container or a fresh

1943

weighing of each material and carried out on separate days. A duplicate assay is a repeat

1944

assay using the same solutions. Since it does not include all the variables of weighing and

1945

dilution, it is not truly independent.

1946

1947

1948

1949

1950

6.3 Participants and their role

1951

1952

The participants may be national control laboratories, relevant manufacturers, academic

1953

or health care laboratories. The supplier of the material may also be a participant. Since the

1954

ultimate purpose of the study is establishment of an international reference standard,

1955

competent laboratories representative of the WHO regions should be included whenever

1956

possible.

1957

1958

Potential participants in the collaborative study should be given an outline of the aims of the

1959

study and a description of the materials to be included. If it is intended that participants use

1960

the same assay method, a protocol for the procedure should be provided and sufficient time

1961

allowed for laboratories to become familiar with the method. Potential participants should be

1962

asked to indicate:

1963

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- 1964 • the assay methods which they could undertake,
- 1965 • whether they could compare the proposed number of materials in each assay ,
- 1966 • the number of assays that they could carry out,
- 1967 • that they are willing to report their raw data using a reporting form supplied.
- 1968 • whether the laboratory operates under an accredited or other quality management system

1969

1970

The presence of a quality system does not

1971

guarantee the quality of the data submitted; the

1972

assessment of the collaborative study data is the

1973

key to data reliability.

1974

1975

Prior to participation in a collaborative study,

1976

participants may be requested to undertake

1977

proficiency studies or tests with control

1978

samples.

1979

1980

1981

1982

1983 Participants should also agree:

1984

- 1985 • to complete their studies within the time frame specified,
- 1986 • to accept responsibility for safe handling and disposal of the materials provided,
- 1987 • to use the materials provided for the purpose of the collaborative study only and not for
- 1988 independent research,

1989

1990

Participants may be requested to sign a material

1991

transfer agreement, agreed between the donor of

1992

a collaborative study sample and WHO, as a

1993

condition of participation.

1994

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1995 • not to publish information on a proposed international reference standard without the
1996 prior agreement of WHO since premature publication before establishment of the material
1997 can cause scientific confusion.

1998 Participants should agree to a provisional plan
1999 for publication of the collaborative study,
2000 including proposed authorships, and conditions,
2001 including provisions for anonymity, under
2002 which raw data from the study may be released
2003 for further analysis

2004

2005 Participants will be asked to provide comments on the draft report on the collaborative
2006 study before its submission to WHO. The participants are listed but the results from each
2007 participant are coded so as to retain anonymity.

2008

2009

2010

2011 **6.4 Materials to be included in the collaborative study**

2012

2013 Materials included in a collaborative study may include, in addition to the candidate
2014 standard(s), other standards in current use, coded duplicate samples, typical samples for
2015 which the standard will be used (to assess commutability), samples that are known to be out
2016 of specification (eg samples that may have failed a quality control test for a key parameter
2017 such as potency), or one or more dilutions of a sample included in the study.

2018

2019 Additional materials included in collaborative studies must be such that all the samples
2020 of a given preparation are within specified limits and stable during the time for the study to
2021 be completed. To avoid introduction of bias, samples should be coded and labelled so that
2022 participants cannot identify materials and their sources or duplicate samples. Where
2023 appropriate materials should be screened for freedom from infectious agents

2024

2025 The materials should be distributed to the participants in accordance with current postal
2026 or air freight regulations. (14). They should be securely packaged and appropriately labelled.
2027 If any materials are frozen, they should be packaged in insulated containers with sufficient

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2028 coolant to last until they are delivered. They should be accompanied by directions for
2029 storage, handling and safe use and disposal. Participants should be requested to report the
2030 condition of the samples immediately upon receipt to the study organizer.

2031

2032 Temperature monitoring devices may be
2033 included with the shipment, or on the label of
2034 the standard.

2035

2036 If concerns about the condition of the samples
2037 are reported, the study organizer should make a
2038 decision as soon as possible whether there is a
2039 need to ship replacement samples, and inform
2040 the participant of the outcome of the decision
2041 concerning the condition of the samples.

2042

2043 **6.5 Reporting of results**

2044

2045 Each participant should be provided with a form in which to provide information on:

2046

- 2047 • the assay method(s) used, including details of the assay design and layout. This may also
2048 include details of the animals (species, strain, weight range, sex, pretreatment and
2049 method of randomization), or of other test materials (for example, organisms, cells, test
2050 kits or substrates);
- 2051 • the nature of diluent solutions and the procedure for making dilutions of test and standard
2052 materials. This is important for the calculation of results and the detection of causes of
2053 variation, bias or inaccuracy;
- 2054 • assay results given as raw (that is, unprocessed) data. All data obtained should be
2055 reported, and an explanation must be given for proposed rejection of any data.

2056

2057 In addition, participants should provide their own statistical calculations for each assay
2058 as this helps to show whether they interpret their results in the same way as the biometrician
2059 who analyses all the results.

2060

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2061 **6.6 Analysis of results**

2062

2063 Results from all participants are analyzed by statistical methods described and
2064 considered appropriate by the biometrician responsible for the design of the study, who
2065 should be experienced in the statistical evaluation of the results of various types of assay.
2066 This analysis requires access to suitable computing facilities and statistical software. Each
2067 assay should be analyzed separately, and as appropriate its validity tested, and the relative
2068 potency and precision calculated (for example, as means and 95% confidence intervals). Any
2069 questions should be discussed promptly with the participant concerned.

2070

2071 The variability in results between assay methods, and between laboratories, should be
2072 described and assessed as part of the analysis. For example, an analysis of variance may be
2073 used to assess the statistical significance of differences between methods and laboratories.
2074 Other possible causes of variation, such as differences between candidate reference materials
2075 when more than one is included, should also be assessed. An assessment should be made of
2076 factors that may be the cause of significant heterogeneity of potency estimates, nonlinearity
2077 and differences in slopes. There is no generic outlier detection rule.

2078

2079 Sources and causes of apparent outliers may not be consistent within
2080 assays, between assays, between laboratories or methods. Omission of
2081 any data should be taken into account in subsequent analysis.

2082

2083 As part of the overall study analysis, for each candidate preparation, the results of all the
2084 assays carried out by each participating laboratory, with each assay method, should be
2085 combined, where appropriate, and the potencies and confidence limits calculated. There is no
2086 generic method for combination of estimates.

2087

2088 The methods to use for combination of estimates depend upon factors
2089 such as the intended use of the standard, information about assay
2090 systems, and the nature of the estimates and their distribution.

2091

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2092 The results of assays should also be displayed graphically, for example
2093 as histograms, as this may help to detect unusual features that may be
2094 overlooked in the study of numerical data alone.

2095

2096

2097

2098 **6.7 Report on a collaborative study**

2099

2100

2101

2102 A copy of the draft report is sent to each participant together with the code used to
2103 identify them. The participants should confirm that:

- 2104 • their data have been correctly interpreted in the analysis;
2105 • the proposed material is suitable to serve as a reference standard for the purpose defined;
2106 • the proposed unitage is appropriate.

2107

2108 The final report, amended where necessary and stating that the participants have agreed
2109 with it, is submitted to WHO. The information to be provided is outlined in Section 7 of these
2110 Recommendations.

2111

2112

2113

2114

2115

Any disagreement should be noted, together
with any relevant critical comments, for further
consideration by the ECBS.

2116 The report of the collaborative study on a proposed international reference standard is
2117 the copyright property of WHO.

2118

2119

2120

2121

2122

2123

2124

The report published by WHO is assigned a BS
document number and is intended for
consideration by the Expert Committee on
Biological Standardization. The study authors
are strongly encouraged to submit a revised
version of the report for publication in a peer-

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2125 reviewed scientific journal. A manuscript
2126 submitted for publication should report the
2127 outcome of the ECBS decision and it is likely
2128 that the data and methods will need to be
2129 presented in a more summarised manner than in
2130 the BS document..

2131

2132 When the reference standard has been established, the report is used as the basis of an
2133 instruction leaflet for users that accompanies every dispatch of the material (a model is given
2134 in Appendix 2).

2135

2136 Data used to support the establishment of an international biological reference standard
2137 are made available to a user of the material either through reference to a scientific publication
2138 on the material or through the report provided to the ECBS to support the request to establish
2139 the material, or both .

2140

2141 The BS document that describes the
2142 report of the study may be placed on the
2143 WHO website

2144

(www.who.int/biologicals)

2145

2146

2147

2148

2149 **7. Detailed information to be provided to WHO**

2150

2151 The following information should be provided in the report to WHO, in support of the
2152 submission of a request for adoption of a candidate preparation as an international biological
2153 measurement standard (IS) by the WHO Expert Committee on Biological Standardization.

2154 The information to be provided to support a proposal to establish an interim Reference

2155 Reagent is given in section 7.6.

2156

2157 **7.1 Introduction**

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2158

2159 The Introduction should explain the background and need for an international reference
2160 standard. It should include:

2161

- 2162 • The name of the substance for which an international reference standard is proposed.
- 2163 • A definition of the substance being measured (the "measurand").
- 2164 • The rationale for the choice of units (IU or SI) being proposed.
- 2165 • If the candidate reference standard is a replacement standard, the rationale for the
2166 approach taken to ensure continuity of the IU.
- 2167 • The way in which the study has been designed to evaluate the fitness of purpose for the
2168 intended use of the reference standard, including where appropriate an assessment of
2169 commutability
- 2170 • Whether the material is needed to standardize products for the prevention, treatment or
2171 diagnosis of disease.
- 2172 • Whether the material is subject to requirements for the manufacture and control of
2173 biological substances, is the subject of a monograph in a pharmacopoeia, and is traded
2174 internationally.
- 2175 • Any recommendation by WHO or a recognized scientific organization that the material
2176 should be prepared.
- 2177 • A review of methods currently used for the assay of similar materials, and rationale for
2178 the choice of methods included in the study.
- 2179 • The aims of the collaborative study and details of the participants.
- 2180 • If a pilot study has been performed, appropriate details on the material used and the
2181 results.
- 2182 • If the reference standard is intended for use in the *in vitro* diagnostics field, the
2183 relationship of the approach used to the principles set out in ISO 17511 (9), where
2184 applicable.

2185

2186 **7.2 Bulk material and processing**

2187

2188 The following information should be provided:

2189

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- 2190 • Description of the bulk material including its source, nature (including information about
2191 the donor/s if relevant) and, where appropriate, its composition. This information may be
2192 supplemented by appropriate literature references or patent information or package
2193 inserts.
- 2194 • Details and results of safety and other chemical, physical and biological testing
2195 performed.
- 2196 • Whether batches of bulk material were combined and, if so, the procedure used.
- 2197 • The composition of the material filled including buffers, diluents, bulking agents, or
2198 stabilizers.
- 2199 • The identifying code of the candidate reference standard.
- 2200 • The address of the facility where the bulk material was processed into final containers. If
2201 subcontractors have been used for any stage of the processing, the identity of the
2202 subcontractor(s) should be provided together with a list of the operations they carried out.
- 2203 • Full details of the processing operations (filling, lyophilization and sealing) and the dates
2204 on which they were performed.
- 2205 • The number of containers used to estimate the precision of fill, the intervals at which
2206 weights were determined, and the results expressed as the coefficient of variation.
- 2207 • Evidence of validation of ampoule integrity after the sealing process
- 2208 • The gas under which the material was sealed, its purity, the method used to determine the
2209 residual oxygen content in the containers and the results obtained.
- 2210 • The method used to determine the residual moisture content in the containers and the
2211 results obtained (as a percentage of the dry weight).
- 2212 • Details and results of other testing performed on the contents of the final containers.
- 2213 • The number of final filled containers in the batch offered to WHO.
- 2214 • The address of the intended place of storage and the name of the present custodian.
- 2215 • The storage conditions including temperature.

2216

2217 **7.3 Stability studies on the product in the final container**

2218

2219 The information should include:

2220

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2221 • The name of the laboratory(ies) that obtained the stability data and details of the assay
2222 method(s) used to obtain them.

2223 • The details of the stability study, including the number of assays carried out and the
2224 details of the samples assayed, including temperatures and duration of storage Results of
2225 assay of the activity remaining in each container after exposure, together with the 95%
2226 confidence intervals.

2227

2228 The methods used for estimation of the 95%
2229 confidence intervals for the predicted percentage
2230 loss of activity per year (28) should be
2231 described.

2232

2233 • An assessment of the stability of the material.

2234

2235 This may be based on the accelerated
2236 degradation studies, in the form of the predicted
2237 percentage loss of activity per year together with
2238 the 95% confidence intervals at the proposed
2239 storage temperature and any other appropriate
2240 temperature (eg +20c and/or +37c) which is
2241 similar to or higher than the expected conditions
2242 of delivery of the reference standards. In some
2243 cases other methods may be appropriate, such as
2244 real-time stability studies where the Arrhenius
2245 equation does not apply.

2246

2247 • An assessment of the stability of the reconstituted reference standard.

2248

2249 **7.4 The report of the collaborative study**

2250

2251 The following information should be provided on the collaborative study:

2252

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- 2253 • The reason why an WHO biological standard is needed and the history of decisions of the
2254 ECBS or of WHO, if any, to support the need for the material
- 2255 • planning and design of the collaborative study and descriptions of the nature of any other
2256 materials included in it.
- 2257 • The assay methods used and which participants used them, but done in such a way as to
2258 maintain blinding so that participants cannot be identified.
- 2259 • For each assay method, the number of assays that each participant was asked to perform
2260 and the number actually carried out.
- 2261 • A description of the statistical analysis carried out, including the way in which the
2262 linearity and parallelism of the dose-response curves were established and any problems
2263 that arose.
- 2264 • Results obtained from the statistical analysis:
- 2265 - The numbers of valid and invalid results,
2266 - the grounds for any exclusion of results (e.g., nonparallelism or nonlinearity),
2267 - a comparison of assay results from materials tested by different assay methods, together
2268 with their interpretation and comments on particular factors, such as the frequency
2269 distribution of the estimates, differences in potency estimates and any observed factors
2270 which may account for these, and any differences observed between different assay
2271 methods
- 2272 - for each laboratory using a given assay method, the within-assay variation and the
2273 overall between-assay variation where possible,
2274 - the overall estimates of relative potencies by each assay method, calculated both with
2275 and without outlying results,
- 2276 The (raw) data should be available on
2277 request to WHO (Secretary, ECBS) for a
2278 period of a least 20 years, or longer if the
2279 standard is still in use.
- 2280
- 2281 • The final figure for the overall estimate of the potency of the proposed reference standard,
2282 comments on the validity of the overall estimate, and if appropriate, the 95% confidence
2283 intervals and the method of deriving them.

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- 2284 • In studies on proposed first international reference standards, an assessment of the degree
2285 to which the calculation of potencies relative to the proposed reference standard reduced
2286 differences between laboratories and between methods.

2287

2288 **7.5 Other information**

2289

2290 The report should also include:

2291

- 2292 • A recommendation for establishment of the material to serve as a reference standard
2293 together with any limitations on its use (e.g., suitability only for certain assay methods),
2294 together with a recommended potency in international or other relevant units.

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The basis of assignment of units to the first IS of a material is the results of the collaborative study. Since the value assigned to the preparation is arbitrary in the case of IU, it may be convenient to propose the value as a rounded number instead of a number derived by statistical analysis of results. For replacement standards, the value proposed should ensure the continuity of the IU.

- 2306 • A formal statement of the traceability path of the International Unit established by the
2307 proposed standard

- 2308 • A consideration of the relationship of the Unit established by the proposed standard with
2309 previous units for the same material, including evaluation of the extent to which
2310 continuity of the IU has been maintained

- 2311 • A formal consideration of uncertainty, including a statement of the uncertainty of content
2312 derived from the variance of the fill, and an evaluation of the requirements of uncertainty
2313 statements in the context of the traceability path

- 2314 • An evaluation of the extent to which commutability has been demonstrated in the
2315 collaborative study.

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- 2316 • A list of the names and addresses of the participants. The coding used to refer to
- 2317 participants in the body of the report should not correspond to the order in which they are
- 2318 listed.
- 2319 • Tables and histograms of the results of the collaborative study.
- 2320 • A summary of the participants' comments on the report.
- 2321 • Acknowledgements, summary and references.
- 2322 • A copy of the proposed instruction leaflet and safety data sheet for users. It is
- 2323 recommended that a consistent format is used to ensure that no relevant information is
- 2324 omitted. A guide is given in the Appendix.
- 2325 • If requested, the detailed manufacturing records including results of in-process controls.
- 2326 • If requested, detailed results of tests performed on the bulk and/or filled material.

2327
2328

2329 **7.6 Report on a collaborative study on a proposed Reference Reagent**

2330 The report on a collaborative study on a proposed Reference Reagent, which may be
2331 submitted for publication in a scientific journal, should include the following information:

- 2332 • Title
- 2333 • Authors
- 2334 • Summary (which includes; the priority need for the material; the number of laboratories,
- 2335 and countries represented, in the collaborative study; the aim of the study; the results; any
- 2336 comments; the stability of the proposed material; a proposal for adoption by the Expert
- 2337 Committee on Biological Standardization that states the code number of the preparation,
- 2338 and the proposed potency)
- 2339 • Introduction
- 2340 • The number of laboratories, and countries represented, in the collaborative study
- 2341 • Materials (which, for the proposed Reference Reagent, should include the information
- 2342 specified in section 7.2)
- 2343 • Stability of proposed interim Reference Reagent
- 2344 • Assay methods
- 2345 • Results (including the statistical analysis)
- 2346 • Discussion/conclusions

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- 2347 • Proposal (for adoption by the Expert Committee on Biological Standardization that states
2348 the code number of the preparation and the proposed potency)
- 2349 • References (if any)
- 2350 • Participants (who, unless it has been agreed to the contrary, are referred to in the body of
2351 the report only by anonymous code numbers, which do not correspond to the order in
2352 which they are listed)
- 2353 • Tables/Figures
- 2354 • Acknowledgements

2355

2356

2357

2358

2359 **8. Establishment of an international biological reference standard**

2360

2361 A preparation may be established as an international biological reference standard by
2362 the WHO when:

2363

- 2364 • The report on the collaborative study has been prepared, all participants have had the
2365 opportunity to comment on the report, and the report together with all comments have
2366 been reported to the ECBS.
- 2367 • Any queries raised by Working Groups or other groups requested by WHO to provide
2368 peer review of proposals have been answered satisfactorily.
- 2369 • All queries raised by members of the Expert Advisory Panel on Biological
2370 Standardization after they have examined the information provided under Section 7 have
2371 been answered satisfactorily, and
- 2372 • the ECBS has come to an agreement based on the evidence provided and the expert
2373 recommendations for the material.

2374

2375 The decision of the Expert Committee is endorsed by the Director-General of WHO.

2376 A list of international biological reference standards is published from time to time in the

2377 WHO Technical Report Series and the current version is available in the WHO web site

2378 (www.who.int/biologicals). Reference standards that have been established or discontinued

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2379 are included in an appendix to reports of meetings of the WHO Expert Committee on
2380 Biological Standardization. Catalogues are also available from custodian laboratories in
2381 printed and electronic form.

2382

2383 **9. Storage and distribution of international biological reference standards**

2384

2385 Custodian laboratories store and distribute international biological reference standards
2386 on behalf of the WHO. The identity of the custodian laboratory for a particular reference
2387 standard is given in the list of reference standards cited above. A key responsibility of the
2388 custodian laboratories is to maintain the integrity of the stored materials. The laboratories
2389 have comprehensive contingency plans to ensure that this integrity is maintained (29).
2390 Custodian laboratories are also encouraged to identify and maintain off-site storage of
2391 sufficient numbers of each WHO International Standard to allow establishment of a
2392 replacement in the event of catastrophic loss of or damage to the entire storage facility.

2393

2394 These include monitoring of sample storage and
2395 alarm systems with protocols and procedures in
2396 place to respond to alerts that are designed to
2397 maintain low temperature storage of the
2398 preparations. Systems are also in place to avoid
2399 accidental or intentional tampering with freezer
2400 or alarm settings. The laboratories have back-up
2401 emergency generators and provide training for
2402 relevant personnel to maintain low temperature
2403 storage of the reference standards.

2404

2405 Custodianship of international biological
2406 reference standards requires considerable
2407 commitment and investment on behalf of the
2408 host institution.

2409

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2410 Custodian laboratories ensure that appropriate precautions are taken to ensure that
2411 shipments of biological reference preparations comply, where appropriate, with international
2412 regulations on transport of infectious substances (14).

2413

2414

2415

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2416 **PART B. GENERAL CONSIDERATIONS FOR THE PREPARATION,**
2417 **CHARACTERIZATION AND CALIBRATION OF REGIONAL, OR**
2418 **NATIONAL BIOLOGICAL REFERENCE STANDARDS**

2419

2420 **1. Introduction**

2421

2422 As supplies of an IS may be limited, regional and national authorities may consider
2423 preparing and establishing secondary reference standards, calibrated against and traceable to
2424 the primary WHO materials, for wider use. Similarly, a manufacturer undertaking the assay
2425 of many batches of a biological product will usually establish a laboratory reference standard
2426 for routine use in these assays. The activities of such secondary preparations should be
2427 calibrated in International Units by direct comparison with the international reference
2428 standard or if necessary by comparison with a regional or national reference standard. The
2429 amount of effort involved in setting up validated secondary reference standards should not be
2430 underestimated. For this reason, countries in a given region are advised to collaborate in order
2431 to prepare regional reference standards. By doing so, the reference standards are likely to
2432 have wider application and duplication of effort is avoided or minimised.

2433

2434 International biological reference standards are distributed free of charge to national
2435 control laboratories and intergovernmental organizations for their intended purpose.

2436

2437 International biological reference standards are
2438 usually not intended for use as working
2439 standards to be used in every assay.

2440

2441 If an international reference standard is not available from WHO, a regional or national
2442 authority may need to establish a reference standard and, if appropriate, define a unit of
2443 activity.

2444

2445 An example is the European Pharmacopoeia unit
2446 of activity for some biological reference
2447 standards

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2480 the batch. It is advisable to monitor stability through an appropriate programme and to

2481 recheck stability from time to time against the relevant international reference standard.

2482

2483 Results of tests with the IS in the context of a

2484 stability monitoring programme are of interest to

2485 WHO, and laboratories are encouraged to report

2486 the results to Secretary, ECBS.

2487

2488 Requirements for labelling should be modified to suit the context in which the material has

2489 been prepared and will be used.

2490

2491 **4. Calibration**

2492

2493 The calibration of a secondary reference material is a complex process and more

2494 extensive guidance than can be provided here is required. Considerations that need to be

2495 taken into account include, but are not necessarily restricted to:

2496

2497 - the higher order reference standards to which the regional or national

2498 standards are traceable to,, which is usually the WHO IS

2499 - compliance with regulatory requirements; calibration of secondary standards

2500 for therapeutic products should comply with local regulatory requirements

2501 whereas calibration of secondary standards for diagnostic use should follow

2502 the principles set out in ISO 17511 (9)

2503 - whether an uncertainty value should be assigned; compliance with the

2504 requirements for metrological traceability will, in many cases involve the use

2505 of restricted or single specified methods of analysis, and statements of

2506 uncertainty of the assigned unitage in terms of the IS, but there are

2507 exemptions as described by ISO 34

2508 - while the range of assay methods may be restricted, calibration will often

2509 involve a very large data set in order to minimise the uncertainty

2510 - how stability should be evaluated; stability is usually carried out using a

2511 monitoring program against the IS, (rather than the predictive model used for

2512 establishment of the IS)

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2513 - the need to verify the calibration obtained.

2514

2515

2516

2517 The number and geographical origin of the participants are likely to be more limited
2518 than for a global collaborative study to establish an IS. In some instances, as few as two
2519 participants, the body intending to establish the material and the supplier of the material, may
2520 be sufficient. Great care should be taken to calibrate secondary reference standards as
2521 accurately as possible in order to avoid systematic bias in the estimation of potency. This
2522 may require a larger number of replicate assays.

2523

2524 Reports on collaborative studies to evaluate secondary reference standards should
2525 comply with the requirements of the organizing body. Final reports should be submitted to
2526 and retained by the organizing body. Instructions for use and safety information should be
2527 supplied with the reference standards to users.

2528

2529

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2569

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Appendix 1

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2698

2699

Considerations for assignment of priorities to development of WHO International Biological Measurement

2700

Standards or Reference Reagents

2701

2702

Based on Annex 3 TRS 904 (7)

2703

Type of standard	Decision point	Guidance	Comments
International Standard	Is the proposed material a replacement or a new standard	A replacement standard generally has a higher priority than a new standard	A higher priority for a new standard can be justified where the material is expected to have a high impact, based on the considerations below
	Is the proposed material to be used to standardize an approved medicine, or an established <i>in vitro</i> diagnostic method, rather than an investigational medicine or investigational method	A candidate standard for an approved medicine or established method generally has the higher priority	A higher priority for a standard for an investigational product or method can be justified where the product or method is in late stage development
	Is the proposed material a potential standard for more than one product or method	A candidate standard for more than one product or method will generally have a higher priority than a product-specific or method-specific standard	
	Is the proposed material to be used to standardize a product or <i>in vitro</i> diagnostic method of public health importance	A candidate standard for a product or method of major public health importance will generally have higher priority than standards for other medical indications	
	Is the proposed material to be used to standardise a product or method of	A candidate standard for a product or method of global importance will	A higher priority for a regional standard can be justified where the

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	global importance	generally have higher priority than standards of regional importance	material is expected to have a high public health impact
Reference reagent	Is the proposed material to be used to standardise a product or method for which the clinical utility is not yet apparent, or methods are not yet agreed	A candidate reference reagent for which an international need exists from both regulatory and scientific considerations will have a higher priority than a reagent for which no such need exists.	

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Appendix 2

2704

2705

2706 **Information to be included in instruction leaflets and safety data sheets for users of** 2707 **international or other biological reference standards**

2708

2709 It is strongly advised that these leaflets and data sheets are prepared in a standard

2710 format.

2711

2712 Publication of instruction leaflets on theWHO

2713 website and also on the website of the custodian

2714 laboratory is encouraged

2715

2716

2717 The package insert or instructions for use accompanying an international biological

2718 reference standard should include the following information:

2719

2720 • The name and address of the custodian laboratory and the distributor if different.

2721 • The name of the reference standard and its identifying code.

2722 • The status of the material (International Standard or interim Reference Reagent) and year
2723 of establishment.

2724 • The defined potency or other parameter, together with a reference to the relevant WHO
2725 Expert Committee and collaborative study reports.

2726 • Citation of the report submitted to the ECBS that supported the establishment of the

2727 standard and citation of any publications in the scientific literature describing the

2728 characterization of the reference standard

2729

2730 The report submitted to the ECBS that

2731 supported the establishment of the standard

2732 may also be distributed together with the

2733 instruction leaflet and safety data sheet.

2734

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- 2735 • Details of preparation of the material relevant to its use that, where appropriate, may be
2736 conveyed to the user with the agreement of the provider of the source material
2737 - details of the nature and formulation of the filled material,
2738 - mean fill volume or mass with number of containers tested and coefficient of variation,
2739 - residual moisture and oxygen with number of containers tested,
- 2740 • Recommended storage temperature and time. Since the distributor has no control over
2741 the conditions under which the reference standard is held after receipt, instruction to use
2742 as soon as possible after receipt is advisable.
- 2743 • Where appropriate, the method of reconstitution with the period of use and storage
2744 conditions after reconstitution .
- 2745 • The intended use of the material.

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For standards intended for use with *in vitro* diagnostic devices, detailed information may be provided, where available, to assist users to document traceability to the reference standard. This may be in the form of a protocol that evaluates the lack of matrix effect in newly developed methods; evaluates the linearity of the reference standards in the system under evaluation; specifies the procedure for transfer of the assigned value of the reference standard to the users calibrators; and provides information to validate the accuracy and the precision of the system under evaluation.

- 2761 • Directions for safe use and disposal of the reference standard before and after
2762 reconstitution.

- 2763 • A statement that the material is not for administration to humans.

- 2764 • Any disclaimers over liability concerning use of the material

2765

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2766 Most of this information is required in the instructions for use of secondary reference
2767 standards.

2768

2769 The following information should be given in a safety data sheet:

- 2770 • The name and address of the custodian laboratory and the distributor if different.
- 2771 • The name of the reference standard and its identifying code.
- 2772 • The status of the material (International Standard or Reference Reagent) and year of
2773 establishment.
- 2774 • The physical nature of the material and, if freeze-dried, a statement that it is hygroscopic.
- 2775 • Any hazards on exposure to the contents of the container.
- 2776 • For material that is potentially infectious, a statement to this effect together with details
2777 and results of testing for infectious agents that has been performed.
- 2778 • For pathogenic material, a statement to this effect.
- 2779 • Instructions for safe handling and disposal, including action to be taken with spillages.
- 2780 • Instructions on action to be taken if someone is exposed to the material by direct contact
2781 including skin contact, ingestion and accidental injection.

2782

2783 This information is required equally for a secondary reference standard.

2784

2785

= = =