

GUIDELINES on REGISTRATION of VACCINES and OTHER IMMUNOLOGICAL PRODUCTS for HUMAN USE in UGANDA

(Version 1 August 2001)

1. GENERAL

Definitions

Immunological products include vaccines, immunoglobulins, antisera and *in vivo* diagnostic antigens.

A *vaccine* is a product intended to stimulate the immune system in the prevention, amelioration or treatment of disease or infection. A vaccine may be a live attenuated preparation of bacteria, viruses or parasites, inactivated (killed) whole bacteria (bacterins) viruses or parasites, living irradiated cells, crude or purified fractions of organisms, including those derived from recombinant DNA in a host cell, synthetic antigens, polyneucleotides (eg. plasmid DNA vaccines), inactivated bacterial toxins (toxoids), or a combination of the above.

Immunoglobulins and *antisera* are preparations of antibodies of human or animal origin respectively intended to treat or provide immediate protection against infections.

A *diagnostic antigen* is a crude or purified fraction isolated from microbial culture and intended for *in vivo* detection of an existing specific immune response (antibodies).

All applications for registration of vaccines and other immunological products should therefore conform to these definitions.

Exemptions

All applications for products shall include information as detailed in this guideline except for the following exemptions:

- a) Products approved for sale by UNICEF shall be exempt from supplying information detailed in sections 7, 8 and 10 below. In such cases the applicant shall provide a WHO product file and reference shall be made in the application form to the relevant sections of the WHO product file.
- b) For products the subject of a World Health Organisation Technical Report Series (WHO TRS) document on the requirements for manufacturing and testing of that particular product, the information in sections 9.1 to 9.6 shall be provided as required in the WHO TRS document. Photocopies of the *relevant sections* of the TRS document shall be sufficient for this purpose. All tests described in the TRS document shall be carried out and the results presented in the application dossier.

Annex 1 lists the WHO TRS documents currently available for vaccines.

General Requirements

- 1.1 All documents are to be submitted typewritten or computer printed in ENGLISH. Where originals are in another language, copies shall be presented together with certified English translations.
- 1.2 Each completed application must contain a complete index to the various appendices and each page of the application dossier must be numbered.
- 1.3 The appropriate application fee shall accompany each complete application form, ie. US\$500 for any product manufactured outside Uganda, US\$ 300 for any locally repackaged foreign product and US\$200 for any product manufactured in Uganda. Subsequent applications to amend any part of the application shall be accompanied by the fee of US\$50 per change.

1.4 Registration procedures shall commence only if Form NDA:R5 with its appendices has been properly completed. Only the information required in the appendices should be furnished.

1.5 All documents shall be addressed to:

The Executive Secretary/Registrar,
National Drug Authority, Plot 46 48 Lumumba Avenue
PO Box 23096, Kampala, UGANDA

Phone: (+256) 41-255665 / 255628

Fax: (+256) 41-255758

Email: nda@imul.com

1.6 Payment of fees can be made by Bank Transfer to:

National Drug Authority Account no: 0240060034201
Stanbic Bank Uganda Limited, Kampala

or by bank draft in favour of National Drug Authority

2. THE APPLICANT

2.1 Application for the registration of a drug shall be made only by:

- a) the patent holder
- b) the manufacturer
- c) a distributor authorised by the manufacturer or patent holder
- d) an authorised Local Technical Representative (LTR) of the manufacturer or patent holder

2.2 The name, physical address, telephone number, fax number, and e-mail address of the applicant shall be provided

3. PARTICULARS OF THE PRODUCT

3.1 **Proprietary name** means the name, which is unique to a particular product and by which it is generally identified (and by which it is registered in the country of manufacture)

3.2 **Approved / INN / generic name** in relation to a drug means the internationally recognised name of such a product or such other name as the NDA may determine

3.3 **Strength** shall be given per unit dosage form, eg. mg/mL, IU/G, IU/mL, etc.

3.4 **Pharmaceutical form** shall mean the form in which the drug is presented, eg. solution, suspension, emulsion, etc. For injections, the type of presentation, eg. vial, ampoule, etc. and the type of content, eg. powder for reconstitution, solution, suspension, oily solution, lyophilised freeze-dried powder, etc. shall also be stated

3.5 **Indication** shall be the intended use of the product

3.6 **Description of the product** shall mean a full visual description of the product including colour, and other relevant features, eg. 'cream coloured emulsion', 'white to off-white freeze-dried plug' etc.

3.7 **Labelling:** the applicant shall ensure that the primary (immediate) packaging of the product is labelled according to the law applicable in Uganda. The following minimum

information shall appear in English on the primary container label:

- a) brand name where appropriate
- b) International Non-proprietary Name(INN)/generic name
- c) quantity of active ingredient per dosage unit
- d) total contents of container
- e) date of manufacture
- f) date of expiry
- g) batch number
- h) storage conditions
- i) name and address of manufacturer

Note: The date of manufacture, address of the manufacturer and storage conditions may be omitted on the primary container if it is a blister or strip pack, or a vial or ampoule less than 10ml, due to lack of space.

The name of the manufacturer may be substituted with a trade mark or other symbol. However all these details shall appear in full on the secondary packaging

3.8 Information leaflet: the product packaging shall include a prescribing information leaflet in the case of prescription medicines, or a patient information leaflet in the case of non-prescription medicines. The leaflet shall include the following minimum information:

- a) International Non-proprietary Name for each active ingredient
- b) Pharmacology: a brief description of mechanism of action and pharmacological effects
- c) Clinical Information:
 - i) indications
 - ii) dosage regimens, including for children
 - iii) contraindications
 - iv) precautions in pregnancy, lactation, renal and hepatic failure, etc
 - v) adverse reactions including frequency
 - vi) clinically significant drug interactions
 - vii) symptoms and treatment of over-dosage
- d) Pharmaceutical Information:
 - i) dosage form
 - ii) strength
 - iii) excipients
 - iv) storage conditions
 - v) shelf life
 - vi) pack size
 - vii) description of product and package
 - viii) name and address of the manufacturer

4. PARTICULARS OF THE MANUFACTURER(S) AND ACTIVITY

The name, physical address, telephone number, fax number, and e-mail address of the manufacturer shall be provided. Where different activities of manufacture of a given product are carried out at different manufacturing sites, the above particulars shall be provided for each site and the activity carried out at the particular site shall be stated, eg.

	Name	Address	Activity
1.	UgaPharma	Plot 4, City Rd PO Box 5445, Kampala, Uganda Tel: 222207	fermentation
2.	T.M. Pharmaceuticals	Plot 73, Government Avenue PO Box 3459, Nairobi, Kenya Tel: 222218	lyophilization + freeze-drying
3.	Goodman Limited	Eastern Industrial Estate GLN, 13LT, London, UK Tel: 235 898 491	packing

Note: A copy of a valid manufacturing licence shall be provided for each site.

5. AUTHORISED REPRESENTATIVE IN UGANDA

A body corporate (company), licensed by NDA to handle medicines, shall be the applicant's local technical representative (LTR) in Uganda with legal authorisation to take full responsibility for the product on behalf of the applicant, and will be answerable to NDA. A copy of the Authority given to the LTR or agent shall be enclosed. Such a body may be:

- (a) a wholesale pharmacy
- (b) a retail pharmacy
- (c) a registered local branch/office of the applicant, in which a pharmacist is employed

6. SIGNATORY

The signatory shall be a registered pharmacist working for and/or authorised by the manufacturer/applicant. The pharmacist's designation and qualification shall be stated.

7. APPENDIX 1 (of Form NDA: R5)

- 7.1 If the drug has been registered in the country of manufacture, the conditions of registration and copy of the registration certificate shall be submitted. If no such registration has been authorised, all relevant particulars with regards to the progress already made concerning the registration of the drug being applied for shall be furnished
- 7.2 A copy of the manufacturing licence of the manufacturer shall be provided
- 7.3 If product is not registered in country of manufacture, a valid explanation must be given

As an alternative to 7.1 and 7.2 above, a *Certificate of a Pharmaceutical Product* shall be submitted under the WHO Certification Scheme on the Quality of Pharmaceutical Products Moving in International Commerce, where the exporting country shall be the country of manufacture and the importing (requesting country) shall be the Republic of Uganda

8. APPENDIX 2 (of Form NDA: R5)

8.1 Composition of the product

- 8.1.1 **Active ingredients:** a list of the active ingredients (immunogens) shall be given and their amount per unit dose shall be stated
- 8.1.2 **Excipients, adjuvants and preservatives:** a list of all excipients, adjuvants and preservatives shall be given, and their amount per unit dose shall be stated. The reason for their inclusion in the formulation shall be stated.

9. APPENDIX 3 (of Form NDA: R5)

9.1 Manufacture of the active raw material

- 9.1.1 The manufacturer of the raw material shall be stated
- 9.1.2 The method of manufacture of the active raw material shall be described including the following:

9.1.2.1 Description of source, specifications and test methods of starting materials

- animal sources
- virus sources (master and working seeds)
- cellular sources
 - microbial cells

- animal cells
 - primary cells
 - cell lines
- genetic constructs and recombinant cell lines
 - host cells
 - gene construct
 - vector
 - final gene construct
 - cloning and establishment of recombinant cell lines
- cell bank system
 - master cell bank
 - working cell bank
 - end of production cells
 - characterisation and testing of cell banks

9.1.2.2 **Growth and harvesting**

- propagation
- harvesting

9.1.2.3 **Purification and downstream processing**

- inactivation (where appropriate)
- purification (where appropriate)
- stability processing
- detoxification

9.1.2.4 **Manufacture of synthetic raw material**

- synthetic peptides
- conjugates and modified active raw materials

9.1.2.4 **In process control specifications and tests** at each stage of manufacture of active raw materials shall be described

9.2 **Raw material specifications and details of their analytical methods** should be given. Where WHO TRS or pharmacopoeial references to specifications and analytical methods are given, full photocopies of those references (monographs) should be supplied. For non WHO TRS, non-pharmacopoeial raw materials, the following minimum information shall be provided:

9.2.1 **Description:** for active (immunogenic) raw materials, the description shall include the biological name (including strain and/or clone designation) or chemical name, the source of the cells from which the raw material is derived, the active components of the cell fractions or purified antigens where applicable, and any chemical modification or conjugation of the immunogenic material. For all raw materials the description shall include the physical state, colour and clarity of the product.

9.2.2 **Specifications and tests** for active raw materials shall be as follows:

9.2.2.1 **Physico-chemical tests**

- identity
- purity
- assay for related proteins and process contaminants(impurities)

9.2.2.2 **Biological activity tests**

- specific identity testing such as Western Blot or ELISA
- cytometric analysis
- neurovirulence testing
- serotyping
- electrophoretic typing
- inactivation studies

- neutralization assays
- titrations
- immunogenicity
- potency

- 9.3 **Comprehensive details of the procedures** involved in the various stages of manufacture of finished product, including packaging shall be given. This shall be in the form of a detailed flow diagram accompanied by a list of equipment used at each stage. Stages of manufacture may include aseptic processing, sterilisation, lyophilisation, freeze-drying and packaging
- 9.4 **Analytical, microbiological and other in-process control procedures** together with the frequency and sequence in which they are carried out during the manufacturing process shall be stated. These processes shall be included in the flow diagram above
- 9.5 **Summarised specifications of the final product shall be given**, ie. the acceptable limits of all the physical, chemical, biological and (where applicable) microbiological parameters. A full description of analytical and other control procedures carried out to ascertain the final product specifications stated above shall be given. Where analytical procedures in various parts of the application coincide, these procedures may be described fully in one part and may be subsequently referred to in other parts, provided that the relevant page and paragraph are clearly identified.

For WHO TRS or pharmacopoeial finished products, photocopies of the relevant monographs may be provided.

For pharmacopoeial finished products where the methods of analysis used are non-pharmacopoeial, detailed analytical validation of such methods shall be provided (see Annex 2 of this guide)

For non-WHO TRS, non-pharmacopoeial (in-house) finished products, the following minimum information shall be provided:

- **Specifications and test methods for all dosage forms**
 - Description
 - Identity: the test method should be proven to be specific and sensitive for active ingredient(s)
 - Potency: the test method should be proven to be specific, sensitive and stability-indicating for active ingredient(s)
 - Impurity limits: to identify and determine the level of degradation products of active ingredients, and active ingredient – excipient interaction impurities
- **Additional specifications and test methods for oral liquids**
 - Uniformity of content and mass
 - pH
 - Microbial limits
 - Antimicrobial preservative content/preservative efficacy test
 - Antioxidant preservative content
 - Extractables from primary container
 - Alcohol content
 - Dissolution for suspensions
 - Redispersibility
 - Specific gravity
 - Water content
- **Additional specifications and test methods for Parenterals**
 - Uniformity of content and mass
 - pH
 - Sterility

- Endotoxins/pyrogens
- Particulate matter
- Water content
- Antimicrobial preservative content/PET
- Antioxidant preservative content
- Extractables
- Functionality of delivery systems, eg. syringeability for prefilled syringes
- Osmolality
- Redispersibility

Note: All tests should be performed unless developmental pharmaceutical studies or process validation prove that they are unnecessary. Such proof should be provided in the application dossier.

9.6 Batch Manufacturing Records (BMR)

Copies of original documents used in the manufacture of one complete batch, ie. from release of raw materials to release of final product for marketing, shall be submitted including QC reports. Batch records for one particular batch should include:

- manufacturer's or supplier's certificates of analysis of raw materials
- adsorption records
- formulation records
- filling records
- lyophilisation records
- packaging records
- labelling records
- reconciliation records
- manufacturer's Certificate of Analysis of the finished product.
- a certified copy of a State Batch Release Certificate for the batch, including results of purity, potency and efficacy testing.

9.7 Specifications of the packaging material

The following information shall be provided:

- A general description of the container and closure system including primary(inner)and secondary(outer) packaging, and other components such as spoons and syringes.
- The chemical identity of materials for each component of the system
- Detailed specifications and tests for primary (immediate) packaging components such as:
 - glass containers
 - plastic containers and closures for solid dosage forms, ophthalmics, parenterals, blood products
 - rubber closures

Such specifications and tests shall be as per the British Pharmacopoeia, European Pharmacopoeia, or United Pharmacopoeia, or in-house, and certificates of analysis shall be provided as proof that the packaging conforms to specifications.

- Evidence of suitability of the container and closure system for the finished product:
 - compatibility of primary packaging components with the finished product
 - performance of the system in drug delivery, eg. actual volumes of teaspoons and eye drop bottles, extractable volumes of vials and ampoules

9.8 Stability studies on finished product

Evidence of stability of the product shall be submitted as follows:

- Should be in market pack (as described in 9.7 above)
- The investigator should be identified and should sign the study report
- The date and location of the studies should be identified
- Type of study should be specified – accelerated, real time

- A detailed protocol should be given
- Summarised results should be provided
- Conclusions should be stated on:
 - proposed storage conditions
 - proposed shelf life
 - in-use storage conditions and shelf life
- Post-approval stability studies commitment should be given (in case shelf-life is not covered at the time of submission of the dossier)
- In general, studies shall be conducted and documented in the following way:
 - **Objective:** The aim of the studies shall be to establish shelf-life and storage conditions appropriate to climatic zone IV
 - **Type of study:** The applicant shall specify whether the studies are real time or accelerated
 - **Protocol:**
 - **Test samples** – from pilot or production batches
 - 2 different batches for stable active ingredient
 - 3 different batches for unstable active ingredient (as per WHO classification)
 - Active ingredients should also be from different batches wherever possible
 - Selection of samples from each batch should be random.
 - **Storage conditions** – for real-time studies
 - Zone I (temperate): 21°C, 45% RH
 - Zone II (subtropical): 25°C, 35% RH
 - Zone III (hot/dry): 30°C, 35% RH
 - Zone IV (hot/humid): 30°C, 75% RH
 - Plus/minus 2 degrees C, plus/minus 5% RH

Notes: i) test conditions must be controlled, ie. in stability chambers and not on open shelves
ii) Real-time studies should last for at least 6 months by the time of dossier submission and should continue to the end of the proposed shelf life (a written commitment should be made by the applicant)
 - **Storage conditions** –for accelerated studies
 - Zones I and II
 - For relatively stable active ingredients: 40±2°C, 75±5 % RH, duration = 3 months
 - For relatively unstable active ingredients: duration increased to 6 months
 - Zones III and IV
 - 40±2°C, 75±5% RH, duration = 6 months
 - **Selection of samples for testing:** a description of the sampling plan used to select samples for storage and subsequent testing from the test batch shall be given
 - **Orientation of containers:** for liquid and semi-solid products samples should be stored in upright, horizontal and inverted positions to ensure full interaction with all primary packaging materials
 - Frequency of testing
 - Accelerated: at 0, 1, 2, 3 and 6 months
 - Real-time: at 0, 6, 12 months, and annually thereafter
 - The end-of-shelf-life specifications with limits shall be stated
 - Analytical tests (all test methods shall be fully described)
 - Potency
 - Content of decomposition products (impurities)
 - Physicochemical properties which are potency indicating
 - Moisture content for lyophilised products

- pH where appropriate
 - Sterility or control of bioburden or preservative efficacy tests
 - Viability of cells after freezing and thawing
 - Pyrogenicity
 - General safety
- Analytical method validation for non-pharmacopoeial potency testing methods shall be provided
 - Where a product is to be reconstituted before use, tests on the stability of the product after reconstitution should also be done and a shelf-life of the reconstituted product determined
 - In the case of multi-dose vials for liquid injectables, tests should be done to determine stability after the first puncturing
- **Presentation of results:**
- Should be both as tables and as graphs
- **Interpretation of results and shelf life determination:** shall be based on the least stable batch:
- A shelf-life of 24 months can be assigned if:
 - the active ingredient is known to be stable (WHO classification)
 - 6-month studies show no significant change (accelerated or real-time) and the applicant has given written commitment to continue the studies and provide results up to at least 24 months
 - A shelf life of greater than 24 months can be granted as long as **real-time studies** are conducted for at least as long as the proposed shelf life and indicate stability of the product
- **Labelling recommendations** shall be stated as follows:
- Store under normal storage conditions (15–30 °C)
 - Store between 2–8°C (refrigeration, no freezing)
 - Store below 8 °C (refrigeration)
 - Store between –5 to 0 °C (in a freezer)
 - Store below –18 °C (in a deep freezer)
- Note:** these recommendations must be reflected on the product samples submitted with the application
- **Retesting**
- Stability studies should be repeated if there are any changes in the formulation, manufacturing method, or packaging material of a product

10. APPENDIX 4 (of Form NDA: R5)

10.1 Toxicology

Particulars of laboratory tests and their conclusions performed to demonstrate all aspects of toxicity of the product, and to predict toxic effects during use, with special reference to:

- Single dose toxicity
- Repeat dose toxicity
- Genotoxicity including mutagenicity
- Carcinogenicity
- Reproductive toxicity
 - Effects on fertility and early embryonic development
 - Effects on embryo-foetal development
 - Effects on prenatal and postnatal development
- Local tolerance (potential for adverse effects at site of administration)

- Reversion to virulence studies for live attenuated vaccines to investigate the possibility of the vaccine reverting its wild, disease causing form (ie. backpassage studies)
- Studies to prove inactivation of killed organism vaccines

10.2 Pharmacology

Particulars of laboratory tests and their conclusions performed to demonstrate all aspects of pharmacology of the product, and to predict mode of action, with special reference to:

10.2.1 Pharmacodynamic studies (in laboratory animals or humans)

- Primary pharmacodynamics – primary action in target system.
- Secondary pharmacodynamics – resultant action in target systems.
- Safety pharmacology – pharmacodynamics in non-target systems leading to side effects
- Pharmacodynamic drug interactions

10.2.2 Pharmacokinetics studies (in laboratory animals and/or humans)

- Absorption, distribution, metabolism, excretion characteristics
- Relationships between pharmacokinetic characteristics and therapeutic and toxic effects
- Pharmacokinetic drug interactions observed or predicted.

10.2.3 Each study shall include the following

- objective
- experimental protocol including methodology and materials
- summarised results and their statistical analysis
- discussion and conclusions

10.3 Particulars of therapeutic effects and indications of the product

The proposed therapeutic use(s) of the product shall be stated. Evidence of potential benefit of use in Uganda shall be provided, in particular, compatibility of the dosing schedule with the National Immunisation Programme

10.4 Particulars of tests which have been performed on humans regarding the efficacy of the drug and the indications for which it will be used (clinical trials). Details of studies on the following subjects shall be provided:

- **Immunogenicity, efficacy and safety studies (in humans) including:**
 - Controlled clinical trials on efficacy (vaccination-challenge studies, field efficacy studies)
 - Studies on potential beneficial interactions with other vaccines of the same type (boosting)
 - Studies on potential decrease in efficacy when administered at the same time as other vaccines (interference)
 - Studies on interchangeability with other vaccines of the same type
 - Local and systemic tolerance studies to determine the maximum tolerable dose
 - Field safety studies
- **Individual clinical studies protocol shall include the following:**
 - Objectives (see classification above)
 - Identity and qualifications of key personnel involved
 - Location(s) of study
 - Dates of study
 - Design
 - Selection of subjects (inclusion, exclusion criteria)
 - Selection of controls
 - Selection of control treatment (if applicable)
 - Number of subjects
 - Response variables (end points)
 - Minimisation of bias (randomisation, blinding, compliance)
 - Treatments given: identity and quality of the investigational and control products used, dosage used, duration of treatment, duration of observation periods, any concurrent treatments and their justification
 - Analytical methods for determining drug concentrations in body fluids, tissues
 - Analysis of results including statistical analysis.
 - Discussions and conclusions on efficacy and safety, including but not limited to:
 - suitability for Uganda in terms of % protection produced
 - suitability for Uganda in terms of the national immunization programme
 - applicability of the clinical studies to the Ugandan population

- adverse reactions observed and their relationship with the administered dose

10.5 Particulars of side effects, contra-indications, etc. of the drug: the adverse effects of the product, the patients in which it should not be used, precautions before or during use in certain patients, shall be stated.

11. APPENDIX 5 (of Form NDA: R5)

- 11.1** References to literature shall be precise, quoting the year of publication and the relevant page(s). Photocopies of relevant literature may be attached
- 11.2** A minimum of two samples of the final product for each package size being applied for must be provided in the form in which it shall appear on the market

Annex 1: WHO TRS DOCUMENTS CONTAINING DETAILS of REQUIREMENTS for BIOLOGICAL PRODUCTS

(as listed in WHO Technical Report Series, NO. 872, 1998 Annex 6)

Requirements for Biological Substances and other sets of recommendations

The specification of requirements to be fulfilled by preparations of biological substances is necessary in order to ensure that these products are safe, reliable and potent prophylactic or therapeutic agents. International recommendations on requirements are intended to facilitate the exchange of biological substances between different countries and to provide guidance to workers responsible for the production of these substances as well as to others who may have to decide upon appropriate methods of assay and control.

Recommended requirements and sets of recommendations concerned with biological substances are formulated by international groups of experts and are published in the Technical Report Series (TRS) of World Health Organisation, as listed here.

Requirements

1. *General Requirements for Manufacturing Establishments and Control Laboratories* Revised 1965, TRS **323** (1966)
Replaced by *Good manufacturing practices for biological products*, TRS **822** (1992) and *Guidelines for national authorities on quality assurance for biological products*, TRS **822** (1992)
2. *Requirements for Poliomyelitis Vaccine (Inactivated)*
Revised 1981, TRS **673** (1987) Addendum 1985, TRS **745** (1987)
3. *Requirements for Yellow Fever Vaccine*
Revised 1975, TRS **594** (1976), Addendum 1987, TRS **771** (1988)
Revised 1995, TRS **872** (1998)
4. *Requirements for Cholera Vaccine*
Revised 1968, TRS **413** (1969), Addendum 1973, TRS **530** (1973)
5. *Requirements for Smallpox Vaccine*
Adopted 1966, TRS **323** (1966)
6. *General Requirements for the Sterility of Biological Substances*
Revised 1973, TRS **530** (1973), Amendment 1995, TRS **872** (1998)
7. *Requirements for Poliomyelitis Vaccine, Oral*
Revised 1989, TRS **800** (1990)
- 8 & 10. *Requirements for Diphtheria, Tetanus, Pertussis and Combined Vaccines* Revised 1989, TRS **800** (1990)
9. *Requirement for Procaine Benzylpenicillin in Oil with Aluminium Monostearate* Revised 1966, TRS **361** (1967), Discontinued 1989, TRS **800** (1990)
11. *Requirements for Dried BCG Vaccine*
Revised 1985, TRS **745** (1987), Amendment 1987, TRS **771** (1988)
12. *Requirements for Measles Vaccine (Live)*
Revised 1987, TRS **771** (1988), Replaced by Requirements No. **47**
13. *Requirements for Anthrax spore Vaccine (Live, for Veterinary Use)*
Adopted 1966, TRS **361** (1967)

14. *Requirements for Human Immunoglobulin*
Adopted 1966, TRS **361** (1967), Replaced by Requirements No. **27**
15. *Requirements for Typhoid Vaccine*
Adopted 1966, TRS **361** (1967)
16. *Requirements for Tuberculins*
Revised 1985, TRS **745** (1987)
17. *Requirements for Influenza Vaccine (Inactivated)*
Revised 1990, TRS **814** (1991)
18. *Requirements for Immune Sera of Animal Origin*
Adopted 1968, TRS **413** (1969)
19. *Requirements for Rinderpest Cell Culture Vaccine (Live) and Rinderpest Vaccine (Live)*
Adopted 1969, TRS **444** (1970)
20. *Requirements for Brucella abortus Strain 19 Vaccine (Live, for Veterinary Use)*
Adopted 1969, TRS **444** (1970), Addendum 1975, TRS **594** (1976)
21. *Requirements for Snake Antivenins*
Adopted 1970, TRS **463** (1971)
22. *Requirements for Rabies Vaccine for Human Use*
Revised 1980, TRS **658** (1981), Amendment 1992, TRS **840** (1994)
23. *Requirements for Meningococcal Polysaccharide Vaccine*
Adopted 1975, TRS **594** (1976), Addendum 1980, TRS **658** (1981)
24. *Requirements for Rubella Vaccine (Live)*
Adopted 1976, TRS **610** (1977), Addendum 1980, TRS **658** (1981)
Replaced by Requirements No. 47
25. *Requirements for Brucella melitensis Strain Rev. 1 Vaccine (Live, for Veterinary Use)*
Adopted 1976, TRS **610** (1977)
26. *Requirements for Antimicrobial Susceptibility Tests*
I. Agar Diffusion Tests Using Antimicrobial Susceptibility Discs
Revised 1981, TRS **673** (1982)
Addendum 1982, TRS **687** (1983), Addendum 1985, TRS **745** (1987)
Addendum 1987, TRS **771** (1988), Addendum 1989, TRS **800** (1990)
Addendum 1990, TRS **814** (1991), Addendum 1991, TRS **822** (1992)
Discontinued 1991, TRS **822** (1992)
27. *Requirements for the Collection, Processing and Quality Control of Blood, Blood Components and Plasma Derivatives*
Revised 1992, TRS **840** (1994)
28. *Requirements for Influenza Vaccine (Live)*
Adopted 1978, TRS **638** (1979)
29. *Requirements for Rabies Vaccine for Veterinary Use*
Adopted 1980, TRS **658** (1981), Amendment 1992, TRS **840** (1994)
30. *Requirements for Thromboplastins and Plasma Used to Control Oral Anticoagulant Therapy*
Revised 1982, TRS **678** (1983)
31. *Requirements for Hepatitis B Vaccine Prepared from Plasma*

- Revised 1994, TRS **858** (1995)
32. *Requirements for Rift Valley Fever Vaccine*
Adopted 1981, TRS **673** (1982)
 33. *Requirements for Louse-Borne Human Typhus Vaccine (Live)*
Adopted 1982, TRS **687** (1983)
 34. *Requirements for Typhoid Vaccine (Live, Attenuated, Ty 21a, Oral)*
Adopted 1983, TRS **700** (1984)
 35. *Requirements for Rift Valley Fever Vaccine (Live, Attenuated) for Veterinary Use*
Adopted 1983, TRS **700** (1984)
 36. *Requirements for Varicella Vaccine (Live)*
Revised 1993, TRS **848** (1994)
 37. *Requirements for Continuous Cell Lines Used for Biologicals Production*
Adopted 1985, TRS **745** (1987)
 38. *Requirements for Mumps Vaccine (Live)*
Adopted 1986, TRS **760** (1987), **Replaced by Requirements No. 47**
 39. *Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques in Yeast*
Adopted 1986, TRS **760** (1987), **Replaced by Requirements No. 45**
 40. *Requirements for Rabies Vaccine (Inactivated) for Human Use Produced in Continuous Cell Lines*
Adopted 1986, TRS **760** (1987), Amendment 1992, TRS **840** (1994)
 41. *Requirements for Human Interferons Made by Recombinant DNA Techniques*
Adopted 1987, TRS **771** (1988)
 42. *Requirements for Human Interferons Prepared from Lymphoblastoid Cells*
Adopted 1988, TRS **786** (1989)
 43. *Requirements for Japanese Encephalitis Vaccine (Inactivated) for Human Use*
Adopted 1987, TRS **771** (1988)
 44. *Requirements for Haemorrhagic Fever with Renal Syndrome (HFRS) Vaccine (Inactivated)*
Adopted 1993, TRS, **848** (1994)
 45. *Requirements for Hepatitis B Vaccines Made by Recombinant DNA Techniques*
Adopted 1988, TRS **786** (1989)
 46. *Requirements for Haemophilus Type b Conjugate Vaccines*
Adopted 1990, TRS **814** (1991)
 47. *Requirements for Measles, Mumps and Rubella Vaccines and Combined Vaccine (Live)*
Adopted 1992, TRS **840** (1994), Note, TRS **848** (1994)
 48. *Requirements for Vi Polysaccharide Typhoid Vaccine*
Adopted 1992, TRS **840** (1994)
 49. *Requirements for Hepatitis A Vaccine (Inactivated)*
Adopted 1994, TRS **858** (1995)
- Requirements for Immunoassay Kits [unnumbered]*
Adopted 1980, TRS **658** (1981)

Annex 2: Guidelines to Analytical Method Validation

1) Types of analytical procedures requiring validation

- a) Identification tests
- b) Quantitative test for impurities content
- c) Limit tests for control of impurities
- d) Quantitative tests for active ingredients assay

2) Analytical performance parameters

- a) Accuracy
- b) Precision
- c) Specificity
- d) Limit of detection
- e) Limit of quantitation
- f) Linearity and range
- g) Ruggedness
- h) Robustness

3) Accuracy

- ▶ Definition: exactness of result obtained by analytical method relative to the true value
- ▶ Expressed as % recovery:

$$\frac{\text{ratio of mean of observed measurements}}{\text{true mean}} \times 100$$

- ▶ Measurements: minimum of three measurements each at three concentrations spanning 50% - 150% of the working range of the method using reference standards
- ▶ Acceptance criteria: recovery should be 98% - 102%.

4) Precision

- ▶ Definition: degree of deviation from mean of observed measurements using the method.
 - Repeatability precision: same instrument, analyst, laboratory and day
 - Intermediate precision: same laboratory and instrument, different analyst
 - Reproducibility: different instrument, analyst, laboratory and day.
- ▶ Measurements: minimum of 3 measurements each at 3 different concentrations within the range, and six measurements at 100% of the expected normal working concentration, using reference standards
- ▶ Acceptance criteria: RSD \leq 2%

5) Specificity

- ▶ Definition: ability of the method to discriminate quantitatively and qualitatively between test substances from related substances.
- ▶ Measurements: resolution between chromatographic peaks of test substances and added impurity for assay methods. Presence of positive control peak and absence of peak for negative control in identification methods
- ▶ Acceptance criteria: chromatogram analysis.

6) Limit of detection

- ▶ Definition: lowest concentration detectable by the method
- ▶ Measurement: chromatography analysis
- ▶ Acceptance criteria: the ratio of the peak to background height (signal:noise ratio) should be at least 3:1.

7) Limit of quantitation

- ▶ Definition: the lowest concentration measurable with precision and accuracy by the method.
- ▶ Measurement: chromatography analysis
- ▶ Acceptance criteria: signal:noise ratio should be at least 10:1

8) Linearity

- ▶ Definition: the ability of the method to produce results that are directly proportional to the actual concentration of test substances within a given range
- ▶ Measurements: minimum of 6 measurements each at 5 different concentrations covering 50% - 150% of expected normal working concentrations.

Plot graph of true concentration (x) versus observed result (y), determine Y intercept and coefficient of regression.

- ▶ Acceptance criteria:
 - Regression coefficient should be ≥ 0.98
 - Y intercept should be at 0.

9) Range

- ▶ Definition: the interval between the lowest and highest concentration at which the method is demonstrated to be precise, accurate and linear
- ▶ Measurement: as above
- ▶ Acceptance criteria
 - Active Pharmaceutical Ingredient (API): 80% -120% of expected test concentration
 - Uniformity of content of Final Product (FP): 70% - 130% of expected test concentration
 - Impurity tests: from reporting level to 120% of the maximum allowable limit.
- ▶ Therefore linearity range should be greater than working range.

10) Robustness

- ▶ The ability to remain unaffected by small variations in instrument conditions.

11) Ruggedness

- ▶ Reproducibility precision.

12) Types of validation tests for different analytical tests

Analytical performance Parameter to be tested	Identification test	Assay of impurity	Limit test for impurity	Assay of active drug
Accuracy	X	✓	X	✓
Repeatability precision	X	✓	X	✓
Intermediate precision	X	✓	X	✓
Reproducibility precision	X	✓	X	✓
Specificity	✓	✓	✓	✓
Limit of detection	X	X	✓	X
Limit of quantitation	X	✓	X	X
Linearity	X	✓	X	✓
Range	X	✓	X	✓