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SUMMARY PROTOCOL FOR DIPHTHERIA, TETANUS AND PERTUSSIS VACCINE (ADSORBED) PRODUCTION AND TESTING

Summary information on final lot

Name and address of manufacturer

_____

_____

_____

Lot No.

_____

Date of filling

_____

Volume of each recommended single human dose

_____

No. of doses per final container

_____

No. of final containers

_____

Expiry date

_____

Detailed information on manufacture and control

Strains, single harvests, bulks

Diphtheria vaccine

Strain

Identity of C. diphtheriae strain used for vaccine production

_____

Reference No. of seed lot

_____

Date(s) of reconstitution of ampoule(s) for

manufacture

_____

Single harvests used for preparing bulk purified toxoid
Last the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity, method of inactivation and yields.
For diphtheria tetanus vaccines, delete “Pertussis” in the title of the protocol and do not fill in sections relating solely to pertussis vaccine.

Bulk purified toxoid
Reference No.

_____
Volume and Lf/ml

_____
Date and result of test for antigenic purity
(Lf/mg of protein nitrogen)

_____

Test of irreversibility
Lf/ml of test toxoid solution

_____
Temperature of incubation of test toxoid

_____
Dates of beginning and end of incubation

_____
No. of guinea-pigs injected, route and date
of injection

_____
Date of end of observation

_____
Result of test

_____

Specific toxicity test
No. of guinea-pigs injected and date of

injection

_____

No. of Lf per guinea-pig and route of injection

_____

Date of end of observation

_____

Result of test

_____

Tetanus vaccine

Strain

Identity of *C. tetani* strain used for vaccine production

_____

Reference No. of seed lot

_____

Date of reconstitution of ampoule(s) for manufacture

_____

Single harvests used for preparing bulk purified toxoid

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests, volumes, results of tests for bacterial purity, yields and method of inactivation.

Bulk purified toxoid

Reference No.

_____

Volume and Lf/ml

_____

Date and result of test for antigenic purity (Lf/mg of protein nitrogen)

_____

Test of irreversibility

Lf/ml of test toxoid solution

_____

Temperature of incubation of test toxoid

_____

Dates of beginning and end of incubation

_____

No. of guinea-pigs injected, route and date of injection

_____

Date of end of observation

_____

Result of test

_____

Specific toxicity test

No. of guinea-pigs injected and date of injection

_____

No. of Lf per guinea-pig and route of injection

_____

Date of end of observation

_____

Result of test

_____

Pertussis vaccine

Strain

Identity of *B. pertussis* strain used in vaccine

Serological type of strain

_____

Reference No. of seed lot

_____

Date(s) of reconstitution of ampoule(s) for manufacture

_____

Single harvests used for preparing the bulk material

List the single harvests and indicate the medium, dates of inoculation, temperature of incubation, dates of harvests,

volumes, results of tests for bacterial purity, yields and presence of agglutinogen, methods and dates of inactivation, and opacity.

Bulk material
Identification

_____

Volume and opacity/ml

_____

Date, results of and medium used in test
for living organisms

_____

Date of test for presence of agglutinogens
1, 2 and 3 and results

_____

Information on blending

Diphtheria toxoid component
Reference No.

_____

Lf/ml

_____

Volume

_____

Tetanus toxoid component
Reference No.

_____

Lf/ml

_____

Volume

_____

Pertussis vaccine component
Reference No.

_____

Opacity units (calculated from opacities of
single harvests)

_____

Volume

_____

Adjuvant
Nature and concentration (Al or Ca in mg/ml)

_____

Volume

_____

Preservatives
Nature and concentration

_____

Volume

_____

Buffer
Nature and concentration

_____

Volume

_____

Tests on final bulk

Reference No.

_____

Date of completion

_____

Volume

_____

Sterility test
Date and result of test

_____

Specific toxicity test
Tetanus and diphtheria (optional)
No. of guinea-pigs injected and date of

injection

_____

Number of single human doses injected per guinea-pig, volume and route of injection

_____

Date of end of observation

_____

Result of test

_____

Pertussis

(i) Mouse weight-gain test

Strain of mice

_____

No. of animals in test group and control group

_____

Date of injection

_____

Volume and route of injection

_____

Date of end of observation

_____

Results of tests on a separate sheet of paper, give all relevant details for mice in the control and test groups (survival, mean weight before injection and three and seven days after it) and indicate percentage weight gain of test group as compared with control group.

(ii) Other tests

Mention here date and results of any other specific toxicity test which may have been performed (e.g., tests for heat-labile toxin, lymphocytosis promoting factor, pertussis toxin on cell cultures and endotoxin).

Potency test

Diphtheris

(i) Tests based on challenge
(*a*) Three-dilution aassays

Lethal challenge
Weight of guinea-pigs

_____

Date of immunization and volume of dilutions administered

_____

Date of challenge

_____

Challenge dose

_____

Date of end of observation

_____

Results

	Dilution	*No. of survivors/No. of animals injected*	*Median effective dose (ED_{50})*	
Reference vaccine (__ IU/ml)	_____	_____		
	_____	_____	_____	ml
	_____	_____		
	_____	_____		
Test vaccine				ml
	_____	_____	_____	
	_____	_____		

Potency of test vaccine is__IU per single human dose. Limits of 95% confidence interval (in %) are____

Multiple intradermal challenge
Report all relevant information on animals, and dates of immunization, challenge and end of observation
Results

	Dilution	*Mean score*
Reference	_____	_____

vaccine (__ IU/ml)	–	_____	_____
		_____	_____
Test vaccine		_____	_____
		_____	_____
		_____	_____

Potency of test vaccine is__IU per single human dose. Limits of 95% confidence interval (in %) are____

(*b*) One-dilution challenge test

Date of performance of last satisfactory three-dilution test

_____

Nature and reference No. of product tested (specify also whether it was a final bulk or a final product)

_____

Provide relevant information validating the one-dilution assay system.

Identity and titre (IU/ml) of reference toxoid

_____

Weight of guinea-pigs

_____

Date of immunization

_____

Date of challenge

_____

Challenge dose

_____

Date of end of observation

_____

Results

	Reference vaccine	*Test vaccine*
Dilution used for immunization	_____	_____
No. of survivors/No. injected	_____	_____

P value indicating the probability that
the test vaccine contains more than
30 IU per single human dose

(ii) Tests not based on challenge

(*a*) Toxin neutralization on cell cultures

Provide separately all relevant information such as identity and titre (in IU/ml) of the reference vaccine, species of animals immunized, dilutions of vaccines, date of immunization, date of bleeding, dilution at which immune sera were assayed, amount of toxin added to dilutions of immune sera and, for each dilution: No. of wells (or tubes) where cells survived/No. of wells inoculated with toxin-antitoxin mixtures or (control) toxin, median effective doses (ED_{50}s), titre per single human dose confidence interval, etc.

(*b*) Other tests.e.g., competitive enzyme-linked immunosorbent assay (ELISA)

Provide separately all relevant information, including data by which the method was validated.

Potency of test vaccine__IU per single human dose. Limits of 95% contidence interval (in %) are_

Tetanus

(i) Tests based on lethal or paralytic challenge

(a) Three-dilution assays

Species and weight of animals

_____

Date of immunization and volume of dilutions administered

_____

Date of challenge

_____

Challenge dose (indicate whether lethal or paralytic)

_____

Date of end of observation

_____

Results

	Dilution	*No. of survivors (or of animals not paralysed)/No. of animals injected*	*Median effective dose (ED_{50})*	
Reference vaccine (__ IU/ml)	_____	_____		
	_____	_____	_____	ml
	_____	_____		
	_____	_____		
Test vaccine				ml
	_____	_____	_____	
	_____	_____		

Potency of test vaccine is_____IU per single human dose.
Limits of 95% contidence interval (in %) are___

(*b*) One-dilution challenge test

Date of performance of last satisfactory three-dilution test

_____

Nature and reference No. of product tested (specify also whether it was a final bulk or a final product)

_____

Provide relevant information validating the one-dilution assay system.

Identity and titre (IU/ml) of reference toxoid

_____

Animal species and weight of animals

_____

Date of immunization

_____

Date of challenge

_____

Challenge dose (specify whether lethal or paralytic)

_____

Date of end of observation

_____

Results

	Reference vaccine	*Test vaccine*
Dilution used for immunization	_____	_____
No. of survivors (or of animals not paralysed)/No. injected	_____	_____

P value indicating the probability that the test vaccine contains more than *40 IU/single does (60 IU if DTP vaccine is assayed in mice)*

_____

(ii) Tests not based on challenge

Provide separately all relevant information on other tests, e.g., competitive enzyme-linked immunosorbent assay (ELISA) or passive haemagglutination, including the data by which the method was validated.

Potency of test toxoid is_IU per single human dose. Limits of 95% confidence interval (in %) are_

Pertussis
Strain, weight and sex of mice

_____

Date of immunization

_____

LD_{50} in challenge dose

_____

No. of colony-forming units in challenge dose

_____

Date of challenge

_____

Date of end of observation

_____

Results

	Dilution	*No. of survivors / No. inoculated*	*Median effective dose (ED_{50})*	
Reference Vaccine (_ IU/ml)	_____	_____		
	_____	_____	_____	ml
	_____	_____		
	_____	_____		
Test vaccine				ml
	_____	_____	_____	
	_____	_____		

Potency of test vaccine is____IU per single human dose.

Limits of 95% contidence interval (in %) are____

Test for residual free detoxifying agent

Detoxifying agent (formaldehyde or glutaraldehyde)

_____

Date of test

_____

Results (in g/l)

_____

pH
Date of measurement

_____

Results

_____

Tests on final product

Identity test
Test for diphtheria toxoid: method, date and results

_____

Test for tetanus toxoid: method, date and results

_____

Test for pertussis vaccine: method, date and results

_____

Sterility test
No. of times the test had to be performed

_____

No. of containers tested

_____

Media and temperatures of incubation

_____

Date of inoculation

_____

Date of end of observation

_____

Result of the (last) test

_____

Potency test
If this test was not performed on the final bulk, indicate this and report the data obtained on the final product in the space provided for potency tests in the "final bulk" section.

Innocuity test *Mice* *Guinea-pigs*
No. of animals _____
_____
Route of injection _____
_____
Volume of injection _____
_____
Date of injection _____
_____
Date of end of observation _____
_____
Results _____
_____

Test for adjuvant
Date of test
_____

Nature and concentration of adjuvant per single human dose
_____

Test for preservative
Date of test
_____
Nature and concentration of preservative
_____

pH
Date of measurement
_____
Result
_____

Inspection of final containers
Date of inspection

_____

Result

_____

Stability test
For each component of the vaccine indicate separately all relevant details and (as a percentage) the calculated losses of potency per year at different temperatures as determined by accelerated degradation tests, and actual titres (with the limits of 95% confidence intervals) after storage for the maximum period claimed for the product at the recommended temperature.

Certification by the manufacturer

Name of head of production (typed)

_____

Certification by person from the control laboratory of the manufacturing company taking overall responsibility for the production and control of the vaccine

I certify that lot No … of diphtheria, tetanus and pertussis vaccine, whose number appears on the label of the final container, meets all national requirements and satisfies Part A of the combined vaccines section of Requirements for Biological Substances No. 8 and 10, revised 1989 and (if applicable) addenda 19____

Signature

_____

Name (typed)

_____

Date

_____