

Development of 1st PAHO Regional Standard for Pertussis Whole Cell Vaccine

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- PAHO recognised the need for a Regional Standard some years ago. At that time the practice was to use US FDA CBER Lot11 but this was not an International Standard, supplies were limited and as CBER no longer performed pertussis potency assays the assurance of stability was uncertain.

Introduction-2

- WHO was encouraging Regions to develop their own secondary standards programme. To produce a completely new standard was difficult and would take years. The obvious solution was to find a source of existing material.
- At that time the current 3rd IS (ampoule code 66/303) was under consideration for replacement by a 4th IS that had been prepared earlier but not established.
- The 3rd IS had been prepared in 1966 and was identical with the 2nd IS although freeze-dried as a separate lot. It was established by WHO ECBS in 1998 (Biologicals (1998) 29:133-136) when the second IS became depleted. Its stability had been confirmed by repeated collaborative studies, including use in a WHO proficiency study on the intra-cerebral potency assay.

Introduction 3

- For establishment of the 4th IS a repeat collaborative study was required which would involve comparison with the 2nd and 3rd ISs.
- The candidate 4th IS (94/532) was prepared in 1994 and compared with the 2nd IS (66/302) in a 1995-1996 collaborative study run by the SSI, Copenhagen.
- Subsequently the stability of the 2nd IS was questioned and the validity of the calibration of the 4th IS not accepted. Later studies showed that the 2nd IS was stable and the candidate IS was valid.

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- In the meantime 66/ 303 (identical with 2nd IS) was established by WHO ECBS as the 3rd IS in 1998 after a collaborative study to confirm stability (WHO TRS 897: WHO ECBS 49th Report, 2000)
- Because of the time lapse, further collaborative study of stability of the candidate 4th IS and comparison of it with the current 3rd IS was needed before it could be considered for establishment ‘ (The ECBS, 55th meeting, 2004).
- The ECBS also suggested that the collaborative study should include regional reference standard(s) if possible.
- A collaborative study was set up accordingly.

This study consisted of two parts:

-Review data from initial study launched in 1995(O1995)

(comparing 94/532 with the Second IS (66/302))

- Study launched in 2005 (N2005)

(comparing 94/532 with the current Third IS (66/303))

Aims of the study (N2005)

- To assess the suitability of the candidate 94/532 to serve as the 4th IS for whole cell pertussis vaccine
- To assign a unitage to the candidate 4th IS
- To compare the activity of the 3rd IS, the candidate 4th IS and the U.S. Lot No.11 (PAHO candidate regional standard)
- To confirm the stability of these preparations by assessing the relationship between them and to show consistency with their nominal unitages based on currently available information

Participants (N2005)

- Laboratories that currently performed Kendrick intracerebral challenge potency assay test on whole cell pertussis vaccine/or whole cell pertussis based combination vaccines.
- A total of 16 laboratories from 14 countries participated
 - Asia (WP/SEA): 7
 - Europe: 6
 - South America: 3

Materials included in the study(N2005)



- **66/303:** Current 3rd IS (a freeze-dried preparation of whole cell pertussis vaccine) established in 1998 (WHO TRS 897, WHO ECBS 49th Report, 2000)
- **94/532:** Candidate for 4th IS. Freeze-dried preparation
- **U.S. Lot No. 11:** then being used as a standard in the PAHO region. Freeze-dried preparation, established in 1994
- **In-house reference preparations:** Participants were also encouraged to include their in-house reference preparation if available.

Criteria for candidate material for whole cell pertussis vaccine

- ***Source of material***
- ***Record of production***
- ***Opacity***
 - Concentrated bulk e.g. Opacity 180 IOU for 3rd IS and 150 IOU for 4th IS candidate
- ***Volume of material required***
 - Depends on freeze-drier capacity, normally enough for 5000-10,000 amps plus handling volume

Examine suitability of the bulk material to serve the purpose

- Identity
- Presence of fims 2 & 3
- Protection assay (potency by Kendrick test)

Trial work for freeze-drying (1)

- ~ 100 amps as trial fill
- Freeze- drying formulation
 - *Stabiliser*
 - e.g. 8% Dextran and 5% glucose for 4IS candidate
 - M/15 Sorensen's buffer for 3rd IS
 - Define the freeze drying parameters
 - Good physical appearance
 - To make sure the freeze-dry system is adequate

Trial work for freeze-drying (2)

Preliminary study to assess the effect of formulation and freeze-drying conditions on the freeze-dried material in comparison to baseline material

General Characterization

- Residual moisture levels
- Residual oxygen content
- Microbial contamination

Preliminary assays of the proposed standard - % activity remaining after freeze-drying

- Normally only one or two labs involved
- The contents of each ampoule are both sufficient and convenient
- To assess comparability of the freeze-dried material and existing standards
- **Preliminary estimate of potency for the proposed standard in terms of International standard/or other standards**
- Short term stability study (accelerated degradation test for 3 or 4 months)

Study Method(N2005)

- Three common preparations distributed to participants in appropriate packaging
- The participating laboratories were asked to perform **Two independent assays** using their own routine procedures, reagents, mice from local sources and analytical methods.
- Laboratories were requested to report their results and all raw data to NIBSC on a standardised form.
- At NIBSC, data were collected, coded and entered into a database for analyses. The study report was prepared and distributed to all participants for comments before submission to ECBS.

Important information for data analysis (1)

Sample preparation sheet (normally in forms)

- Name of laboratory and assay number
- For each sample, the preparation of dilution should be clear

	Vol. of reconstituted IS or vol from dilution A,B,C	Vol. of diluent	Immunisation vol/per mouse	Resulting dose (IU/dose) or dilution factor of original sample
A (First dilution)				
B (Second dilution)				
C (Third dilution)				
D (Forth dilution)				

Important information for data analysis (2)

Assay sheet (1)

- Name of laboratory and assay number
- Raw data from each assay should be presented for each sample

Vaccine	Dose	IU or dilution factor of original sample/dose	Number of mice/group	Number of mice dead 72 hours after challenge	Number of mice alive 14 days after challenge
	A				
	B				
	C				
	D				

Important information for data analysis (3)

- Assay sheet (2)
 - Name of laboratory and assay number
 - Raw data from each assay should be presented for control of challenge

Control of Challenge	Dose	Number of organisms per dose	Number of mice/group	Number of mice dead 72 hours after challenge	Number of mice alive 14 days after challenge
	1				
	2				
	3				
	4				
	5				

Other Information

- Calculations by participants
- Questionnaire for the assay
 - Mouse
 - Preparation of challenge strain
 - In-house reference
 - Criteria used for assay validity
 - Other comments?

Results

Comparison of dose-response lines

Analysis of the individual assays did not indicate any significant differences among the preparations

Study O1995

- Duplicate ampoules of 94/532
paired t-test ~ 0.7
- Slopes for 94/532, IS2
paired t-test > 0.3

Study N2005

- Slopes for 94/532 and IS3
paired t-test > 0.3

Timeline

- Materials distribution: March 2005
- Data collection: January 2006
- Study report: June 2006
- Report submission to ECBS: July 2006

Stability Study

- June 2003: a number of ampoules of 94/532 were stored at: 56°C, 45°C, 37°C, 20°C, 4°C
- Potency assessed in several Kendrick tests by NIBSC using ampoules stored continuously at -20°C as the reference

<i>Time</i>	<i>Temp.</i>	<i>Potency</i>
1 year	37 °C	1.18
19.5 months	4°C	1.05
19.5 months	20°C	1.01
32.4 months	20°C	1.05

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Results

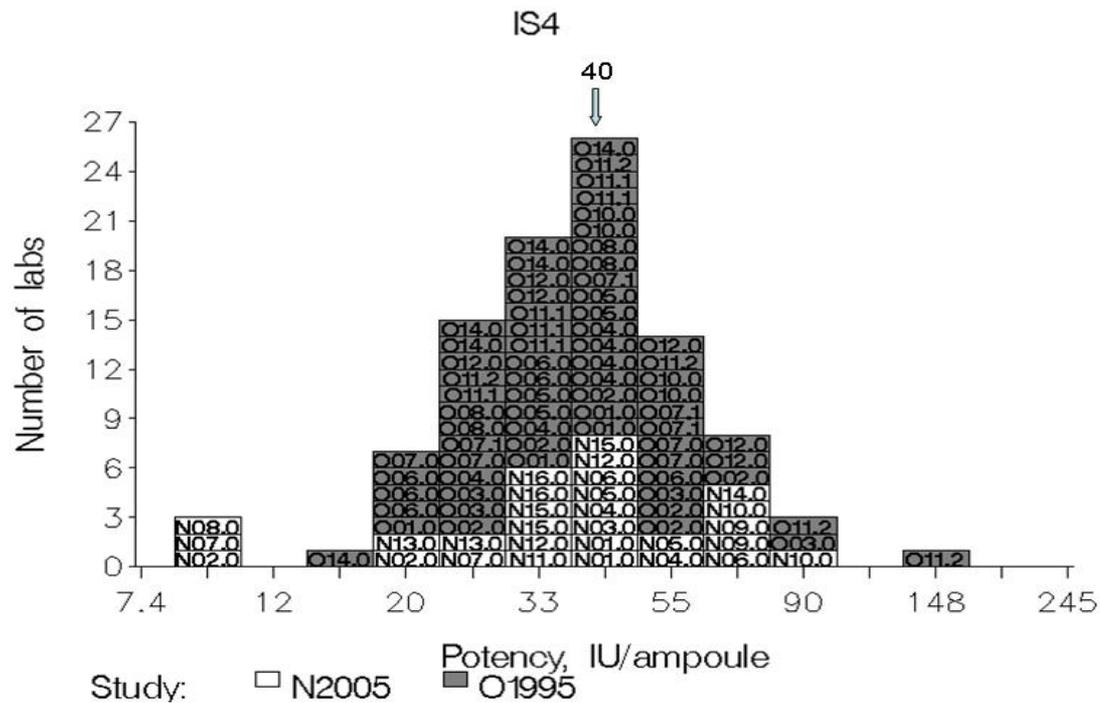
Calibration of the Candidate Standard 94/532



Reference used	Results (IU/amp.)	Study
1 st IS	29.6(25.4-34.5)*	O1995
2 nd IS	39.3 (36.3-42.5)	O1995
3 rd IS	41.2(35.5-47.8)	N2005
2 nd & 3 rd IS (combined)	39.7 (37.0-42.5)	O1995 & N2005

*Consistent difference in slopes of 94/532 or 2nd IS compared with 1st IS

Histogram showing the distribution of estimates of potency for 94/532



Conclusions and Recommendations

- Analysis of the data gave a consistent calibration of 40 IU (37-43)/ampoule for 94/532 in terms of 2nd IS & 3rd IS
- Available data indicated that 94/532 is sufficiently stable to serve as an international standard
- On the basis of the results of the evaluation and with the agreement of the participants in the study N2005, it is recommended that 94/532 be established as the 4th IS for whole cell pertussis vaccine and be assigned an activity of 40 IU/ampoule.

Consequence for 3rd IS

- As the 4th IS replaced the 3rd IS, the latter was dis-established by WHO
- This left NIBSC with >1000 ampoules in store.
- These were offered to PAHO as a candidate Regional Standard and were accepted. They were shipped in two lots to Venezuela for re-labelling, storage and distribution
- As the material had been through numerous collaborative studies and stability confirmed by the most recent one, WHO did not require re-calibration of the Regional Standard