EVALUATING THE QUALITY OF VERO 76 CELL BANK NATIONAL REFERENCE STANDARD USED FOR QUALITY CONTROL OF VACCINES AND BIOLOGICALS PRODUCTS

Nguyen Thi Van Quynh*, Pham Van Hung, Doan Huu Thien, Nguyen Hoang Tùng, Trieu Thanh Hai, Nguyen Thi Mai Huong, Nguyen Khanh Ly, Pham Thi Thu Phuong, Nguyen Thu Quynh, Le Thi Kim Thuy

[*] *National Institute for Control of Vaccine and Biologicals*

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Abstract: The Master cell bank (Vero 76 NICVB/MCB01-18) and Working cell bank (Vero 76 NICVB/WCB01-19) are produced from international standard Vero cells ATCC 76 (ATCC CRLM- 1587TM) was studied and evaluated for quality according to the standards of the World Health Organization (WHO) including criteria such as (identification; sterility; homogeneity; viability; *Mycoplasma*; Retrovirus; tuberculosis bacteria; extraneous agents in vitro and in vivo). The research results show that the cell banks Vero 76 NICVB/MCB01-18 and Vero 76 NICVB/WCB01-19 meet the quality requirements according to WHO TRS 978, part 3, 2013 and WHO TRS 932 part 1, 2006 for National Reference Standard used for research and quality control of vaccines and biological products.

Keywords: Vero 76 cell bank; MCB; WCB, Quality control

1. Introduction

Vero cells originate from the kidneys of adult African green monkeys (Cercopithecus) and were discovered by Y. Yasumura and Y. Kawakita at Chiba University - Japan and published in the journal Nippon Rinsho 21:1209 in 1963. Vero cells are one of the most common mammalian continous cell lines used in research. Additionally, Vero cells have been licensed in the United States to produce both live virus vaccines (rotavirus, smallpox) and inactivated virus vaccines (polio), and worldwide Vero cells have been used to produce several other viral vaccines, including rabies vaccine and Japanese encephalitis vaccine [11], [12].

Vietnam is a country with a manufacturing of vaccines and medical biologicals, and the national vaccine management agency of Vietnam has met WHO standards since 2015. Therefore, proactively setting up secondary standards includes (vaccine standard, antisera, standard toxin, virus or bacterial strain, standard for cell bank, etc.) are called national standards or in-house standards established by the manufacturer and use international standard samples to assess the quality or standardize with secondary standards. One of the standard samples is a standard of cell bank for use in research and quality control of vaccines and medical biological products in accordance with

* Corresponding author
E-mail address: vanquynh1074@gmail.com
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the regulations of the National Vaccine Administration (NRA). according to WHO recommendations [1], [2], [13].

Vero 76 NICVB/MCB01-18 and Vero 76 NICVB/WCB01-19 cell banks are produced from international standard Vero 76 ATCC 76 cells (ATCC CRLM-1587TM) that are evaluated for quality according to WHO standards including : Sterility test; *Mycoplasma*; Tuberculosis bacteria; extraenous agents in vivo and in vitro; Retroviruses; identification; viability; homogeneity between cell tubules.

2. Methods

2.1. Research design

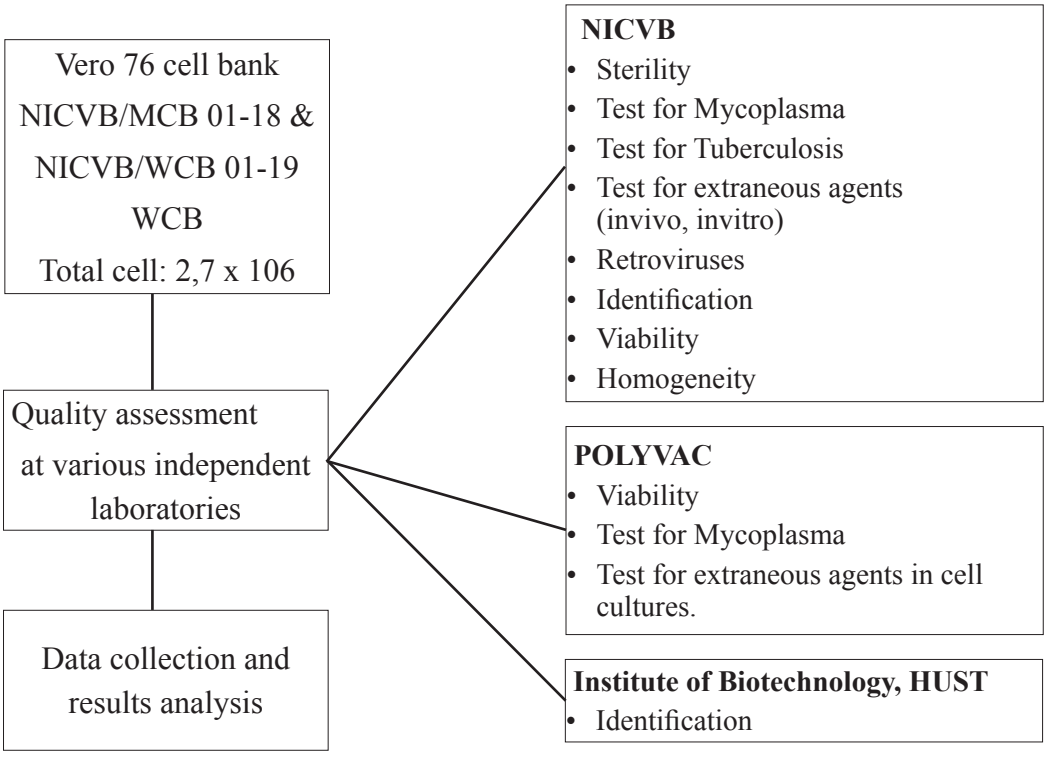

Figure 1. Overview of the study

Techniques used to assess cell bank quality: Sterility test [6]; Test for extraneous viruses [1], [2]; Mycoplasma test [1], [7], [8], [10]; Viability [1]; TB bacteria [1], [3]; Homogeneity [1], [3].

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2.2. Subjects of study

The research subjects are Master cell bank (Vero 76 NICVB/MCB01-18) and working cell bank (Vero 76 NICVB/WCB01-19) cultured and produced at the National Institute of Vaccine Control and Medical Biologicals.

2.3. Research time and location

Research time was conducted from September 2019 to October 2020.

Location: Department of Experimental Environment, National Institute for Accreditation of Vaccines and Medical Biologicals and Institute of Biotechnology, HUST and Labo Center for Research and Production of Vaccines and Biologicals - POLYVAC.

2.4. Materials and equipment

Standard and test samples: Vero cells of ATCC 76 (ATCC CRLM-1587TM): 02 tubes (Lot No: 58494142); Human lung diploid cell MRC5 (P25), ATCC CCL-171: 02 tubes; Rabbit kidney cells RK13 (P174) ATCC CCL-37: 02 tubes; African green monkey kidney cells Vero (P30) ATCC CCL-81: 02 tubes; DNA strain *Mycoplasma pneumoniae* ATCC 29342: 01 tube of 10µl; *M.pneumoniae* ATCC 15531 (F1) : 01 tube; *M.orale* ATCC 23714 (F1) : 01 tube.

Media, chemicals: MEM medium, DMEM, Fetal calf serum (FBS), PBS 1X, Trypsin solution, Tripsin LE, Fungizon, Kanamycin, NaHCO3, 7.5%, L-glutamine, TSA, PPLO.

Equipment: hood laminar Bio II A, Telstar; Incubator (36±1)˚C, with CO2; Olympus Reversing Microscope Beckman Refrigeration Centrifuge, Refrigerator -80˚C Sanyo; -20˚C Kawachi Refrigerator; Refrigerator 2-8˚C; water bath; Thermomo pH meter; Analytical balance; EVOOS 5000 fluorescence microscope; PCR machine ABI Fast 3500 (USA) and some other equipment.

2.5. Data analysis

Using Microsoft excel software to analyze the results. Calculate the ±2SD analyze and evaluate the results.

3. Results

The Vero 76 NICVB/MCB01-18 and Vero 76 NICVB/WCB01-19 Cell Bank are derived from international standard referent by ATCC, Vero 76 ATCC CRLM-1587™ as shown in Figure 2, studied and evaluated quality according to WHO standards with criteria of sterility, viability, Mycoplasma, tuberculosis, homogeneity, in vivo and invitro extraneous agent testing, specific identification, Retrovirus for specific results as follows

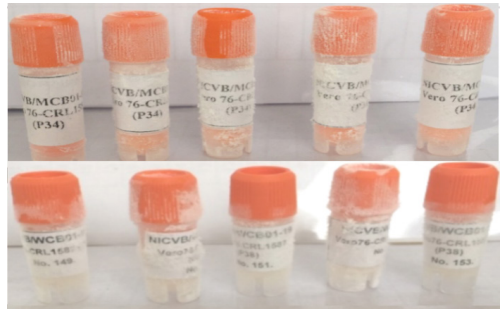


Figure 2: Vero 76 Cell Bank NICVB/ MCB01-18 & NICVB/WCB01-19

3.1. Sterility test results

The results of the Vero MCB and WCB sterility tested on growth medium to detect bacteria and fungi after 14 days of follow-up gave satisfactory results as shown in Table 1.

Table 1: Vero 76 cell bank sterility test results

No.	Sample	Method	Criteria	Result
1	NICVB/MCB01-18	Cultured on Thioglycolate and Soybean medium	No bacteria and fungi detected after 14 days	**Passed**
2	NICVB/WCB01-19	Cultured on Thioglycolate and Soybean medium	No bacteria and fungi detected after 14 days	**Passed**

3.2. Results of Mycoplasma testing

a. PCR method

Vero 76 NICVB/MCB01-18 and Vero 76 NICVB/WCB01-19 cell banks were tested for specific *Mycoplasma* by PCR with 16S rRNA gene specific primers of 229bp in size, refer to the procedure in scientific journals. (Audrey Jean et al. pone.0172358 February 22, 2017) with a sensitivity of 10 copies method gives results as shown in Figure 3.

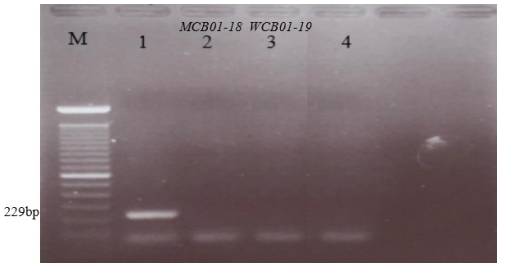


Figure 3: Vero 76 cell bank Mycoplasma determination results NICVB/MCB01-18 & NICVB/WCB01-19.

(M: DNA marker size 2000bp; 1: Vero 76 ATCC-CRL 1587; 2&3: Vero 76 NICVB/ MCB01-18 & NICVB/WCB01-19; 4: H2O negative control)

b. Direct culture method

Table 2: Mycoplasma test results of Vero 76 MCB and WCB cell bank

No.	Sample	Method	Criteria	Result
1	**NICVB/MCB01-18**	Cultured on PPLO	No featured colonies were detected and no color change in liquid PPLO medium was detected after 28 days of supervision	**Passed**
2	**NICVB/WCB01-19**	Cultured on PPLO		Passed

The results of Figure 2 and Table 2 show that banks samples Vero 76 NICVB/MCB01-18 and Vero 76 NICVB/ WCB01-19 met the requirements for *Mycoplasma* test.

3.3. Test results for tuberculosis bacteria

Result of tuberculosis tested on bacteria was performed using the culture method on Lowenstein Jensen specific medium, resulting in Vero 76 NICVB/ MCB01-18 & NICVB/WCB01-19 bank meeting the criteria for testing tuberculosis bacteria as Table 3.

Table 3: Test results for tuberculosis bacteria in Vero 76 MCB and WCB cell banks

No.	Sample	Method	Criteria	Result
1	**NICVB/MCB01-18**	Cultured on Lowenstein Jensen	No tuberculosis bacteria were detected	Passed
2	**NICVB/WCB01-19**	Cultured on Lowenstein Jensen	No tuberculosis bacteria were detected	Passed

3.4. Results of extraneous agents testing in Vero cell bank in vivo

a. Results of extraneous agents testing on 1-day-old sucking mice

Test results for detecting extraneous virus from Vero 76 cell bank NICVB/MCB01-18 & NICVB/WCB01-19 in 1-day-old mice with intra-abdominal injection dose of 0.1ml (10^7 cells/ml)/ sucking mouse x group of 10 test mice and 10 control mice. Follow-up after 28 days showed results as shown in Table 4.

Table 4: Results of extraneous agents test on 1-day-old sucking mice

No.	Sample	Criteria	Result	Conclusion
1	**Control group**	There were no dead mice. 100% are healthy with no signs of disease	100% (10/10) are alive and healthy with no signs of disease	Passed
2	**Vero 76 NICVB/ MCB01-18**	≥ 80% are alive and healthy with no signs of disease	100% (10/10) are alive and healthy with no signs of disease	Passed
3	**Vero 76 NICVB/ WCB01-19**		100% (10/10) are alive and healthy with no signs of disease	Passed

b. Test results for adventitous agents in adult mice

Test results for detecting extraneous virus from Vero 76 cell bank NICVB/MCB01-18 & NICVB/WCB01-19 in adult white mice (15-20g/mice) with an intrapertoneal route dose of 0.5ml (10^7 cells/ml)/mouse x group of 10 test mice and 10 control mice gave results as shown in Table 5.

Table 5: Results of extraneous agents test on adult mice

No.	Samples	Method	Criteria	Result		Conclusion
				After 28 days of observation	**Weight gain compared with the control group (%)**	
1	**Control group**	No injections	100 % are alive and healthy with no signs of disease	100% (10/10) are alive and healthy with no signs of disease	230,63	**Passed**

2	**Vero 76 NICVB/ MCB01-18**	Inject dose of 0.5 ml (10^6 cells/ml)/intraperitoneal route/ 10 mice 15-20g	≥ 80% are alive and healthy with no signs of disease	100% (10/10) are alive and healthy with no signs of disease	96,13	**Passed**
3	**Vero 76 NICVB/ WCB01-19**			100% (10/10) are alive and healthy with no signs of disease	85,70	**Passed**

The results showed that 100% of adult mice were healthy, gained weight and had no signs of pathology during the follow-up period and the results of mouse surgery at the end of 28 days after injection of the test sample, 2 cell banks Vero NICVB/MCB01-18 & NICVB/WCB01-19 the average weight gain of were 96.13% and 85.70% respectively compared with the control group, meeting the requirements of in vivo foreign agent testing in adult mice.

c. Test results for adventitous agents in Guinea-pigs

Test results for detecting extraneous virus from Vero 76 cell bank NICVB/ MCB01-18 & NICVB/WCB01-19 in guinea-pigs (weighing 350-400g/mice) with an intrapertoneal route dose of 5ml (10^6 cells/ml)/mice x group of 10 test mice and 10 control mice and observed for 42 days gave results as shown in Table 6.

Table 5: Results of extraneous agents test on Guinea-pigs

No.	Samples	Method	Criteria	Result		Conclusion
				After 28 days of observation	After 28 days of observation	
1	**Control group**	No injection	100 % are healthy with no signs of disease	100% (10/10) are alive and healthy with no signs of disease	170,0	**Passed**
2	**Vero 76 NICVB/ MCB01-18**	Injection dose of 5 ml (10^6 cells/ml)/ intraperitoneal route/x10 guinea-pig 350-400 gram	≥ 80% are alive and healthy with no signs of disease	100% (10/10) are alive and healthy with no signs of disease	135,8	**Passed**
3	**Vero 76 NICVB/ WCB01-19**			100% (10/10) are alive and healthy with no signs of disease	126,4	**Passed**

The results showed that 100% of the guinea- pigs in both the sample and the control group were healthy, gained weight and showed no signs of pathology

during the 42-day observation and the results of the guinea-pigs surgery at the end of observation showed no signs of pathology, the average weight gain of cell banks Vero 76 NICVB/MCB01-18 and NICVB/WCB01-19 was 135.8 % and 126.4 % respectively compared with the control group, meeting the requirements of extraneous agent testing in Guinea pigs.

d. Test results for adventitous agents on embryonic chicken eggs

Test results for detecting extraneous virus from Vero 76 cell bank NICVB/MCB01-18 & NICVB/WCB01-19 on embryonated chicken eggs with a cell suspension dose of 0.2ml (106 cells/ml)/egg x 10 eggs and 10 eggs as control and observed for 5 days gave results as shown in Table 7.

Table 7: Results of extraneous agents test on embryonic chicken eggs

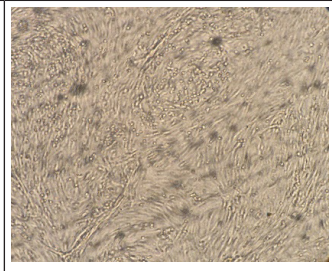
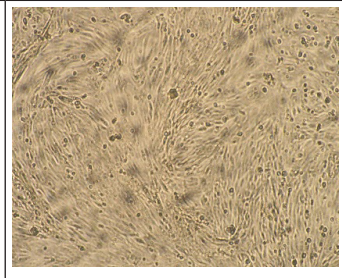
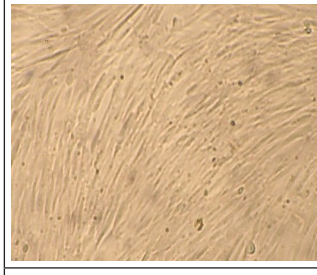
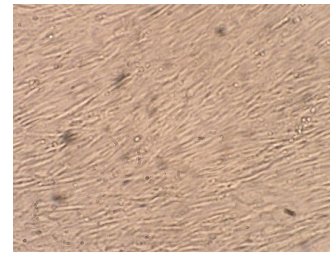
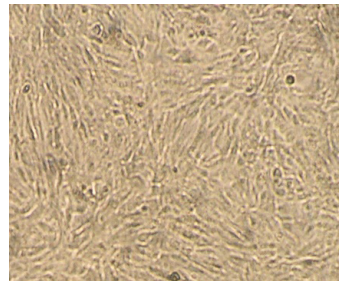
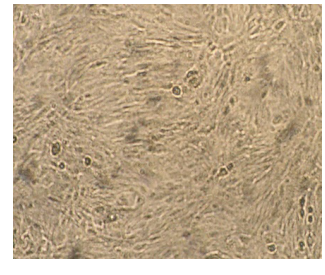
No.	Samples	Method	Criteria	Result	Conclusion
				After 5 days of embryonated infection	
1	**Control group**	No Injection	100% of chicken embryos were alive and no haemagglutinins with chicken red blood cells	100% (10/10) of chicken embryos were alive and no haemagglutinins with chicken red blood cells	**Passed**
2	**Vero 76 NICVB/ MCB01-18**	Injection dose of 0,2 ml (10^6 cells/ml)/ allantoic cavity/10 embryonated checken's eggs	≥ 80% of chicken embryos were alive and no haemagglutinins with chicken red blood cells	100% (10/10) of chicken embryos were alive and no haemagglutinins with chicken red blood cells	**Passed**
3	**Vero 76 NICVB/ WCB01-19**			100% (10/10) of chicken embryos were alive and no haemagglutinins with chicken red blood cells	**Passed**

The results showed that the Vero 76 NICVB/MCB01-18 and NICVB/WCB01-19 cell samples both had 100% alive chicken embryos and no chicken erythrocyte agglutination after 5 days of embryo infection. The control group resulted in 100% live and non-agglutinated chicken embryos proving the test was valid.

3.5. *Results of test for extraneous agents of Vero 76 cell bank NICVB/MCB01-18 & NICVB/WCB01-19 in vitro*

Testing for extraneous agents in the Vero 76 cell bank NICVB/MCB01-18 & NICVB/WCB01-19 in vitro was performed on 3 cell lines such as RK13 ATCC CCL-37, Vero ATCC CCL-81 and MRC5 ATCC CCL-171 after inoculating supernatant of Vero 76 NICVB/MCB01-18 & NICVB/WCB01-19 and 14 days of obsavation gave results as shown in Table 8.

Table 9: Results of test for extraneous agents invitro

<table>
<tr><th rowspan="2">Test</th><th rowspan="2">Criteria</th><th rowspan="2">On cells</th><th colspan="2">Results/sample</th></tr>
<tr><th>NICVB/MCB01-18</th><th>NICVB/WCB01-19</th></tr>
<tr><td rowspan="6">Test for extraneous agents in cell cultures</td><td rowspan="6">No CPE and no virus adsorbs erythrocytes in the cell.</td><td rowspan="2">RK13</td><td>  </td><td>  </td></tr>
<tr><td colspan="2">There were no CPEs in infected RK13 cell bottles after 14 days of observation.
No virus adsorbed erythrocytes in the test sample</td></tr>
<tr><td rowspan="2">MRC5</td><td>  </td><td>  </td></tr>
<tr><td colspan="2">There were no CPEs in infected MRC5 cell bottles after 14 days of observation.
No virus adsorbed erythrocytes in the test sample.</td></tr>
<tr><td rowspan="2">Vero ATCC CCL-81</td><td>  </td><td>  </td></tr>
<tr><td colspan="2">There were no CPEs in infected Vero cell bottles after 14 days of observation.
No virus adsorbed erythrocytes in the test sample.</td></tr>
</table>

3.6. Results of viability test of cell bank Vero 76 NICVB/MCB01-18 & NICVB/WCB01-19

Viability tested results were performed by counting the number of viability units of Vero cells/ total cells in tube, the test was repeated 6 times with results as shown in Table 9.

Table 9: Results of viability test of Vero 76 cell bank

No	Sample	Method	Criteria	Average result of 6 tests
1	**Vero 76 NICVB/ MCB01-18**	Counting live cells and cell cultures	≥80% of cells in cryopreservation (≥ 1,6 x10^6/ml/tube)	**2,01 x 10^6/ml**
2	**Vero 76 NICVB/ WCB01-19**		≥80% of cells in cryopreservation (≥ 1,2 x10^6/ml/tube)	**1,50 x 10^6/ml**

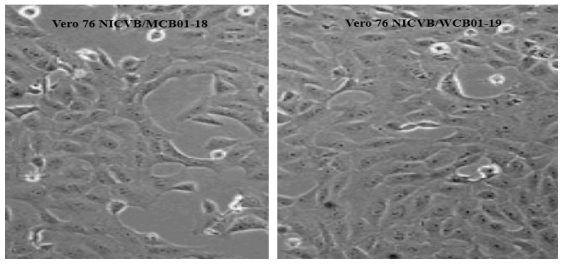


Figure 4: Results of typical morphology of Vero 76 MCB and WCB cells bank cultured in MEM10%FBS medium

The results of Table 9 and Figure 4 show that the vitality is satisfactory. Characteristic image of Vero 76 cells when observed on optical microscopy when cultured on MEM10%FBS.

3.7. Retrovirus test results

The results of Retrovirus agent of Vero 76 NICVB/MCB01-18 & Vero 76 NICVB/ WCB01-19 cell bank gave the results as shown in Figures 5 & 6.

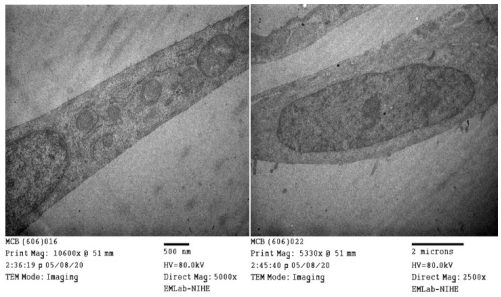


Figure 5: Vero 76 NICVB/MCB01-18 Retrovirus test results

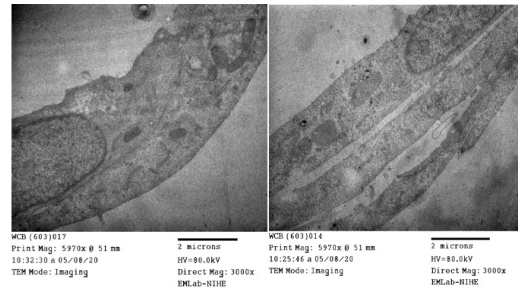


Figure 6: Vero 76 NICVB/WCB01-19 Retrovirus test results

Our Retrovirus test results in cell bank samples: Vero 76 NICVB/MCB01-18 & Vero 76 NICVB/WCB01-19 cells had normal structure, no microorganisms including Retrovirus were observed in the cell sample.

3.8. Results of testing the homogeneity of Vero 76 NICVB/MCB01-18 & NICVB/ WCB01-19 cell bank.

Homogeneity testing was conducted to determine the concentration of cell tubes at the beginning, middle, and end of the Vero 76 NICVB/MCB01-18 & NICVB/ WCB01-19 cell bank tube filling stage. At each stage the test was repeated 6 times, equivalent to 6 randomly taken cell tubes, giving results as shown in Table 10.

Table 10: Results of homogeneity of Vero 76 cell banks

Tests		Vero 76 NICVB/MCB01-18		Vero 76 NICVB/WCB01-19	
		Criteria (10^6/ml)	Results (10^6/ml)	Criteria (10^6/ml)	Results (10^6/ml)
Begin of tube division	1	Cells count within ±2SD (1,86-2,18 x10^6cells/ml)	2,3	Cells count Within ±2SD (1,2 -1,89 x10^6 cells/ml)	1,57
	2		2,20		1,44
	3		1,95		1,50
	4		2,06		1,85
	5		2,20		1,68
	6		1,90		1,40
Midle of tube division	1		2,30		1,56
	2		2,10		1,66
	3		1,95		1,75
	4		2,20		1,56
	5		2,00		1,28
	6		2,10		1,44
End of tube division	1		2,08		1,80
	2		2,01		1,40
	3		1,90		1,75
	4		1,90		1,56
	5		2,20		1,28
	6		2,00		2,3
Xtb		2,02x10^6(cells/ml)		1,54x10^6 (cells/ml)	

Table 10 results show that the cell tubes in the Vero 76 NICVB/MCB01-18 cell bank gave an average result of 2,02 x10^6 cells/tube/ml and NICVB/WCB01-19 gave an average result of 1,54x10^6/tube/ml and the cell tubes at the priod of begin - middle - end of tube division all achieved results within ±2SD, meeting the requirements for homogeneity between cell tubes.

3.9. Quality assessment results of Vero 76 NICVB/MCB01-18 and Vero 76 NICVB/WCB01-19 cell bank at Polyvac

Vero 76 NICVB/MCB01-18 and Vero 76 NICVB/WCB01-19 cell banks were test for viability, *Mycoplasma*, extraneous agents on cell lines at POLYVAC Lab giving results as Table 11.

Table 11: Quality results of Vero 76 MCB and WCB cell bank

Tests	Method	Criteria	Results	
			Vero 76 NICVB/ MCB01-18	Vero 76 NICVB/ WCB01-19
Viability	Count the cell viability /total cells	≥ 80% (≥1,6 x 10^6 cells/ml/ tube)	2,00 x 10^6cells/ml	1,76 x 10^6cells/ml
			1,84 x 10^6cells/ml	1,76 x 10^6cells/ml
			1,81 x 10^6cells/ml	1,60 x 10^6cells/ml
			2,20 x 10^6cells/ml	1,56 x 10^6cells/ml
			1,98 x 10^6cells/ml	1,48 x 10^6cells/ml
			2,10 x 10^6cells/ml	1,48 x 10^6cells/ml

Mycoplasma	PCR	No *Mycoplasma*	**Passed**	**Passed**
Extraneous agents on HEp2C	Culture on HEp2C cells	No cytopathic effect (CPE), no extraneous agents	**Passed**	**Passed**
Extraneous agents on Vero	Culture on Vero cells in another lot	No cytopathic effect (CPE), no extraneous agents	**Passed**	**Passed**
Extraneous agents on MA104	Culture on MA104 cells	No cytopathic effect (CPE), no extraneous agents	**Passed**	**Passed**

Results in Table 11 show that the Vero 76 NICVB/MCB01-18 and Vero 76 NICVB/WCB01-19 cell banks established by NICVB meet quality standards for viability, *Mycoplasma* and extraneous agents.

3.10. Quality assessment results of Vero 76 NICVB/MCB01-18 and Vero 76 NICVB/WCB01-19 cell bank at the Institute of Biotechnology, Hanoi University of Science and Technology.

Vero 76 NICVB/MCB01-18 and NICVBWCB01-19 cell banks were identified by PCR to amplify the target gene of the psi beta and delta globin intergenic region of the green monkey kidney (Cercopithecus aethiops) to design primers for amplification of a 307 bp DNA fragment.

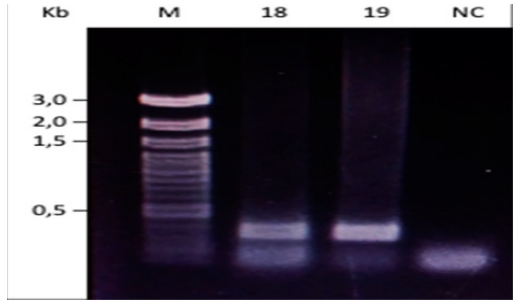


Figure 9: PCR product electrophoresis.

M: standard 100bp DNA; 18: Vero 76 MCB 01-18; 19: Vero 76 WCB01-19; NC: negative control sample

DNA sequences obtained from two samples Vero 76 NICVB/MCB01-18 & Vero 76 NICVB/WCB01-19 were compared with the Vero 76 sequence on Genbank

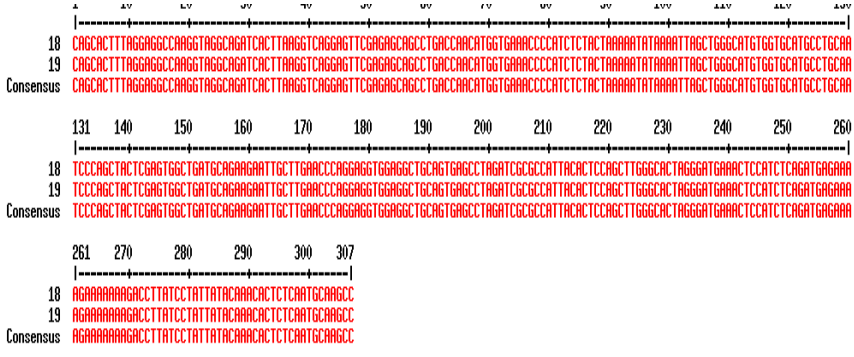


Figure 10: Sequencing results and comparison with Vero 76 on Genbank/NCBI

(18: NICVB/MCB01-18; 19: NICVB/WCB01-19; Consencus: Vero 76 checked on NCBI/ Genbank)

The results showed that the DNA sequences of each target fragment of the psi beta and delta globin intergenic region of green monkey kidney cells from Vero 76 cell bank samples NICVB/MCB01-18 & NICVB/WCB01-19 were 100% similar when Compared with the Vero 76 cell sequence on Genbank/NBCI and met the requirements for specific identification.

4. Discussion

The Vero 76 cell bank NICVB/MCB01-18 and NICVB/WCB01-19 meet quality requirements according to WHO TRS No. 978, 2013 and European Pharmacopoeia 10 Vol 1 [1], [3]. Other results of the evaluation of the quality of Vero 76 cell banks NICVB/MCB01-18 and NICVB/WCB01-19 performed at independent laboratories gave the following results: Pass specific identification by PCR. The psi beta and delta globin gene sequences of the research results are similar to the method of Almeida et al. and Vincent-Falquet J et al. [4], [5], [9]. Passed the requirements of sterility test by culture method on growth medium under appropriate conditions according to WHO and Vietnam Pharmacopoeia V [6].

Regarding viability after production, using the cell counting method and culturing in MEM10%FBS medium results in typical Vero cell morphology when observed on a microscope after culture, achieving the bank viabitity rate. Vero 76 cell bank NICVB/MCB01-18 is 2.01 x 106 cells/tube/ml and NICVB/WCB 01-19 is 1.5 x106 cells/tube/ml. The results showed that the cell banks Vero MCB and WCB had average viability and each test result was ≥ 80% compared to after filling tubes and met the requirements for viability according to WHO standards. TRS No. 978, 2013 [1].

The results of testing the uniformity between cell tubes were conducted to check the number of cells at the beginning - middle - end of the cell bank tube filling process, to prove the homogeneity of cell concentration between tubes in the cell bank, to ensure stability through each use of the cell standard referent bank used in testing vaccines and medical biological products. From the results of Table 10, it shows that Vero 76 NICVB/MCB01-18 and NICVB/WCB01-19 cell banks meet the requirements for homogeneity between tubes according to WHO TRS No. 978, 2013 and European Pharmacopoeia [1], [3].

Test results to detect Mycoplasma in Vero 76 NICVB/MCB01-18 and NICVB/WCB01-19 cell banks by PCR method with synthetic primers of 229 bp 16S rDNA gene gave negative results and were similar to Research by authors Audrey Jean et al. and Frydenberg et al [7], [8] and direct culture methods all showed that Vero 76 NICVB/MCB01-18 and NICVB/WCB01-19 cell banks met the requirements for Mycoplasma testing [10].

Testing the Vero cell bank extraneous agents in vivo including (sucking mice, adult mice, guinea pigs and 10-11 day old embryonic chicken eggs) gave the results No foreign pathogens causing infectious diseases were detected during the culture and tube closure process, meeting WHO standards [1], [2].

Test results for TB bacteria in the Vero 76 cell bank NICVB/MCB01-18 and NICVB/WCB01-19 by culture method on Lowenstein Jensen medium gave negative results, meeting WHO TRS No 978, 2013 standards. and European Pharmacopoeia 10, Vol 1 [1], [3]. In addition, at independent testing laboratories are the POLYVAC

and the Institute of Biotechnology, Hanoi University of Science and Technology, Vero 76 NICVB/ cell bank. MCB01-18 and NICVB/WCB01-19 were evaluated for quality according to the following criteria: Survival, Mycoplasma and foreign agents on HEp2C, MA104 and other Vero cell lines; Specific identification by sequencing of specific target genes psi beta and delta globin of the African green monkey kidney cell species „Cercopithecus aethiops“ gave satisfactory results according to WHO TRS No 978, 2013 and of the European Pharmacopoeia 10, Vol 1 [1], [3].

5. Conclusion

On based our results of study shown that tha Vero 76 NICVB/MCB01-18 and Vero 76 NICVB/WCB01-19 cell banks meet quality requirements for sterility and Mycoplasma; Tuberculosis bacteria, specific identification, viability; homogeneity between cell tubes; not contaminated with advetitious agents invivo, invitro, retrovirus and is used in research and quality control of vaccines and medical biological products in Vietnam according to WHO TRS 978, part 3, 2013 and WHO TRS 932 part 1, 2006.

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