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CLEANING VALIDATION OF A MULTIPURPOSE TANK USED FOR TYPE B *HAEMOPHILUS INFLUENZAE* AND MENINGITIS A AND C VACCINE FORMULATION

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ABSTRACT

The cleaning procedure of a multipurpose tank was validated to ensure the proper removal of waste products and cleaning agents according to regulatory requirements that define acceptable contamination and cross-contamination levels. The analytical methodology chosen to monitor the level of contamination was the measurement of total organic carbon content (TOC); this is a non-specific technique that allows the quantification of organic residues before and after the cleaning procedure. To complete this cleaning validation, a worst case scenario for the contaminant was selected, and the strictest criteria were followed in order to demonstrate cleanliness. Residue recovery tests were performed using swabs and also by rinsing water on specific sampling positions. The results show that the cleaning procedure for this 316 L stainless steel tank was effective in the removal of *Haemophilus influenzae* type B and Meningitis A and C vaccines residues to acceptable levels. Furthermore, the undetectable levels of the sanitizing solution used for cleaning the shared tank, which is used to formulate both vaccines, supports the possibility of using the same reactor to formulate both vaccines without cross-contamination.

Key words : Validation, *Haemophilus influenzae,* Meningitis A and C, Multipurpose tank, Pharmaceutical product, Vaccine.

INTRODUCTION

According to the World Health Organization (2006), the objective of a cleaning validation is to confirm that equipment is clean, with waste products and cleaning agents at acceptable levels, to prevent possible contamination or cross-contamination. After cleaning, the equipment must be stored in a dry condition, and at least three consecutive repetitions of the cleaning procedure must be performed to successfully validate the cleaning procedure (Anvisa, 2010). According to WHO (2006), the ideal validation should include the combination of these two methods. The final evaluation of the cleaning process must take into account the lowest level of waste or product recovery already performed rather than the average value of previous recoveries. Also according to WHO (2006), recoveries greater than 80% are considered good, between 50% and 80% recovery is considered reasonable and less than 50% is considered questionable. Sanchéz (2006)

introduced several guidelines from regulatory agencies that make the process of cleaning validation easier. According to Brazilian legislation, recoveries above 75% are desirable.

Limulus Lysate Amebocyte (LLA) is used for the quantification of bacterial endotoxins from Gram-negative bacilli, among others, by the method of gel formation. Gel formation indicates the presence of endotoxins in the sample with equal or greater amounts compared to the LLA (2003).

Total organic carbon (TOC) is an example of a non-specific method used to measure the concentration of organic carbon; the carbon measured by this method may not only be due to the presence of organic components, but may also be from the cleaning agent itself (Leblanc, 2000). Three criteria are frequently used to determine the limits of contamination acceptability (Anvisa, 2006):

1st Criterion – Presence of no more than 0.1% of contaminant in the maximum dose. Steps must be followed to ensure the following criterion:

Step A: Maximum acceptable limit (μg) of contaminant in the subsequent product (Equation 1).

$$A = \frac{0.0001 \times MTD_{CONT} \times MBS_{SUBS}}{M_{AX}TD_{SUBS}} \quad (1)$$

Where: 0.0001 = Safety factor for injectable products; MTD_{CONT} = Minimum daily dose of the contaminant (μg); MBS_{SUBS} = Minimum size of the subsequent batch (g or mL); $M_{AX}TD_{SUBS}$ = Maximum daily dose (g or mL).

When the MTD_{CONT} is not known, such as for the cleaning solution, the No Observed Effect Level expression (NOEL) can be used, which replaces "0.0001 x MTD_{cont}" from Step A (Equation 2):

$$NOEL = \frac{LD_{50} \times 70}{2000} \quad (2)$$

Where: LD_{50} = Amount of a product that, when taken in a single dose, leads to death in 50% of exposed animals or humans (mg/kg); 70 = Average weight of an adult person (kg); 2000 = Empirical constant.

Step B: Acceptable limit of the contaminant product in the area (μg/cm^2) (Equation 3).

$$B = \frac{A}{SRSA} \quad (3)$$

Where: A = Maximum acceptable mass (μg) of the contaminant in the subsequent product (Calculated from step A); SRSA = Area shared by the products in the same vessel (cm^2).

Step C: Acceptable concentration of the contaminant in the sample (μg/mL) (Equation 4).

$$C = \frac{B \times Area}{Volume} \quad (4)$$

Where: B = Acceptance limit of the contaminant product in the area shared by both products, calculated in step B; Area = the total area when using rinsing water and in the case of the swab technique, Area = the sampled area (cm^2); Volume = the total volume used for rinsing when using rinsing water and when using swab technique, Volume = the volume used to recover the swab (mL).

2nd Criterion - Presence of no more than 10 µg/mL of the contaminant present in the product. In this case Step A becomes the following (Equation 5):

$$A = 10 \times MBS_{SUBS} \quad (5)$$

Where: 10 = Limit of acceptance of 10 µg/mL and MBS_{SUBS} = Minimum size of the subsequent batch (g or mL). Steps B and C are calculated in the same way as for the 1st criterion.

3rd. Criterion - Visual inspection must be performed to detect rough contamination present in small areas that could have been missed by sampling and analysis (visible residues).
All of these criteria apply to possible contamination from both waste products and cleaning agents.

MATERIALS AND METHODS

2.1. Model of the worst contamination possible (Worst case scenario)

Solubility is a parameter that can be used to help determine the worst case scenario because the less water-soluble a product is, the more difficult it is to remove. The cleaning validation was performed in a multipurpose tank where two vaccines are formulated, *Haemophilus influenzae* type b (Hib) and the polysaccharide vaccine meningitis serogroups A and C (Meningitis A and C). To determine which of these two vaccines would result in worse contamination, the solubility of the product in purified water was tested. Purified water was used for the cleaning procedure, followed by collection of material from the tank using a coupon that had been loaded with 200 µL of Hib vaccine and oven-dried for 24 hours at 56º-58º C. After this period, the coupon was removed from the oven; when it reached room temperature, it was added to 150 mL of purified water and mixed at 180 rpm on a stir plate. After 30 seconds, the coupon and the stripping solution were separated and the TOC of the extracted solution was tested. Negative (without Hib vaccine addition) and positive (with 200 µL Hib vaccine added directly to the purified water) controls were performed. All tests were performed in triplicate. The same procedure was repeated for the Meningitis A and C vaccine. Data was analyzed using Equation 6:

$$\%_{rec} = \frac{V_{rec} - C_{neg}}{C_{pos} - C_{neg}} \times 100 \quad (6)$$

Where: $\%_{rec}$ = Percent recovery; V_{rec} = TOC quantified; C_{neg} = Average TOC quantification in the negative control; C_{pos} = Average TOC concentration in the positive control.

The vaccine type with the smallest percent recovery was considered to be the worse contaminant because it would remain more attached to stainless steel tank and would be more difficult to remove.

2.2. Acceptance Criteria

Calculations - The acceptance criteria for the residue from the waste product and cleaning agent was calculated according to Equations 1-5 for each individual sample.
Acceptance criteria for samples of final rinse water, condensate water and water for injectables (WFI) must fit within the limits presented in Table 1.

Table 1
Acceptance criteria for samples of final rinse water, condensate water and WFI

Parameter	Final Rinse Water or WFI Condensate Water	WFI
pH	5.0 - 7.0	5.0 - 7.0
Conductivity at 25°C(µS/cm)	<1.3	<1.3
TOC (µg/L - ppb)	According to the acceptance criteria described	< 500
Maximum endotoxin concentration (EU/mL)	< 0.250	< 0.250

To demonstrate the depyrogenization of the tank, the final endotoxin concentration in the water must be reduced by three logarithmic units compared to its initial concentration, (i.e., a 1000X reduction). Thus, it is necessary that the initial concentration of residual product recovered from the tank before the cleaning process is greater than 250 EU/mL and after this process the concentration must be smaller than 0.250 EU/mL.

Recovery of the vaccine product

The product recovery for the worst contaminating case (Hib vaccine) was performed by sampling with swabs and collecting the rinse water. The contamination was measured, and the percent recovery was calculated; this data is used to evaluate the residual product after cleaning. In order to determine the recovery factor for rinsing, a test was performed using a metallic coupon. The amount of product used was calculated in step B (described in the Introduction section), with the area equal to 25 cm^2, the total area of the coupon.

A 200-µL solution of vaccine that contains the same amount of product previously calculated was prepared. A 200 µL volume was chosen because it is the ideal amount of product to contaminate an area equivalent to 25 cm^2. In this test six coupons were used; five coupons were loaded with 200 µL of Hib vaccine solution and dried for at least 24 hours at 56- 58°C, and the sixth coupon was used as negative control. After this period, coupons were removed from the oven and allowed to reach room temperature. To evaluate the residue recovered from the rinsing technique, the same method described for defining the worst case scenario was used, however, the contact time was increased to 5 minutes because this is the time actually used to rinse the tank during the vaccine formulation process. Positive and negative controls were also performed, as previously described; however, a contact time of 5 minutes was used. Equation 6 was used to calculate the product percent recovery.

In order to determine the recovery factor using the swab technique, five 25 cm^2 pieces of 316 L stainless steel (the same material as the tank) were contaminated with a known amount of Hib vaccine and tested. An uncontaminated piece of stainless steel was used as negative control. Each surface of 25 cm^2 was covered with 200 µL of Hib vaccine solution. The plate was dried for at least 24 hours at 56 – 58 °C. After this time, the plate was removed from the oven and allowed to reach room temperature. All pieces were tested as follows: sampling was performed using two swabs per surface area. The first swab was soaked in a flask containing 20 mL of WFI prior to performing the sampling. A second dry swab was then passed over each sample. Each sample was collected by swabbing the area in the following directions, in order: from the top toward the bottom, from left to right, from right to left, from bottom toward the top and, finally, zig-zag from left to right and vice-versa. The two swabs were soaked in a flask containing 20 mL of water. Inverting the swabs at least 10 times helped to homogenize the solution. TOC analysis was then performed in triplicate.

Positive and negative controls of the TOC measurement were also performed. For the negative control, a vial with 20ml of water and two clean swabs soaked in it were used. For the positive control, 200 µL of Hib solution was added directly to the 20 mL of water in a flask

containing two swabs. TOC analysis was then performed in triplicate. To evaluate the recovery factor, the average percent recovery was calculated for each run for both rinsing and swab techniques. The smallest percent recovery was used as the final result.

Evaluation of the recovery of the cleaning agent

To assess the percentage recovery of the cleaning agent, pH analysis was performed because this is the most widely used method to determine the presence of residual cleaning agent after cleaning the tank. The criterion that selects a 10 µg/mL concentration is the most restrictive, admitting up to 3.512µg/mL NaOH. To model cleaning agent recovery, five coupons were loaded with an amount of NaOH solution calculated in step B and multiplied by an area of 25cm^2. This amount of NaOH was prepared in 200 µL of solution and used to load each coupon. The NaOH was then immediately extracted from the coupon by soaking in water. This method was also used to define the worst-case scenario; a contact time of 5 minutes was used. A positive control was also performed by adding 200 µL of NaOH solution directly to the rinse water and soaking for a contact time of 5 minutes.

Tank cleaning and sampling

The Hib vaccine was used as the endotoxin to contaminate the tank. The cleaning procedure was performed after waiting for the maximum time that the tank could remain dirty, which is 72 hours. After this time, the initial bacterial endotoxin concentration was determined by filling the tank with WFI (sample A). The tank was pressurized and the sample was withdrawn from the bottom. The tank and its removable parts were then rinsed with purified water (purified water - PW) for about 5 minutes. After this rinsing, a 0.5 mol/L NaOH solution was sprayed into the tank and onto the removable parts every 15 min for 1 hour (four NaOH rinses total). Next, the tank and the removable parts were rinsed with PW for 5 minutes. This was followed by an additional rinsing with WFI at 90 °C for 4 minutes and 30 seconds. The last wash time is usually 5 minutes, but to simulate the worst-case scenario in order to validate the cleaning process, this time was decreased by 10%. Operators that perform this procedure are properly trained and wear surgical gloves and suitable uniforms. After cleaning, the tank was re-assembled and filled with WFI at 90 °C, and the sampling procedures were performed using swab and rinse water collection techniques.

For the swab technique, three sampling positions were selected for the determination of TOC (quantification of product samples - B): bottom, side and collecting device. Two swabs were used for each position. The first swab was soaked in WFI from a 20 mL bottle, and then the sampling was performed on the point following the same procedure previously described (item 2.3). Sampling with a second dry swab was performed in the same way. After sampling, each swab was soaked in the same vial with 20 mL of WFI. For the rinsing technique, the tank was filled with WFI to determine TOC content (quantification of product), pH, conductivity (sample C) and bacterial endotoxin (sample D). The tank was pressurized and the sample was withdrawn through the collecting device.

The tank was then left alone to simulate the maximum time until the next use. After this time, new samples were taken for analysis by swab (sample G) and rinsing (sample H - TOC, conductivity, pH and sample I - endotoxin) using the same method as after cleaning. Immediately after sampling, the tank was sterilized with steam. After the sterilization, the tank was sampled again by the rinsing technique (sample L - TOC, conductivity, pH and sample M - endotoxin). This procedure was used to check the interference of the steam used for sterilization and to detect any residue that might have detached from the walls after this process.

The percent recovery from WFI samples were tested immediately after rinsing the tank, using separate bottles for determination of TOC, pH, conductivity and bacterial endotoxin, as follows: After cleaning – Sample E (TOC, conductivity and pH) and Sample F (endotoxin); After the holding time of the clean tank – Sample J (TOC, conductivity and pH) and Sample K (endotoxin); After sterilization – Sample N (TOC, conductivity and pH) and Sample O (endotoxin).

RESULTS AND DISCUSSION

3.1. Choice for the product to model the worst contamination - Worst Case Scenario

Tables 2 and 3 show the recovery values obtained for each vaccine. Table 1 indicates product concentrations ranging from 21.5 to 22.8 µg/mL for the Hib vaccine and from 16.2 to 16.8 µg/mL for the Meningitis vaccine. This indicates that the Hib vaccine showed lower recovery (93.0%) in comparison to Meningitis vaccine. Thus, this cleaning process is further evaluated using the Hib vaccine. This vaccine's active form is a purified capsular polysaccharide from type B *Haemophilus influenzae* and is conjugated to a tetanus toxoid, which may be a more contaminating substance in comparison to the Meningitis vaccine. These results clearly indicate that the Hib vaccine can be considered the worse contaminant compared to the Meningitis vaccine.

Table 2
Summary of TOC measurements obtained for the Hib Vaccine

Hib Vaccine	1st. Run	2nd. Run	3rd. Run
Product (µg/mL)	22.4	22.8	21.5
Negative control (µg/mL)	0.287	0.287	0.287
Positive control (µg/mL)	23.1	23.1	23.1
Percent average recovery	96.9	98.7	93.0

Table 3
Summary of TOC measurements obtained for the Meningitis A and C vaccine

Meningitis A and C Vaccine	1st. Run	2nd. Run	3rd. Run
Product (µg/mL)	16.5	16.8	16.2
Negative control (µg/mL)	0.201	0.201	0.201
Positive control (µg/mL)	16.5	16.5	16.5
Percent average recovery	100.2	102.0	98.4

3.2. Calculation of the acceptance criteria for the product residue and cleaning agent for each sampling technique used

For the waste product, the most restrictive criterion is the one that is limited to 0.01% of the contamination within the dose limit, which is 0.0007 mg/mL (polysaccharide) for rinsing and 0.006 mg/mL polysaccharide for swab. These limits are above the detection limit of the method (0.0004 mg/mL polysaccharide for the residue), which validates this measurement method for use in this cleaning validation. A linear regression ($y = 567.21 x + 0.087$, $R^2 = 0.9919$) was used to convert these values to TOC concentration in order to directly compare the TOC measurements obtained for the samples collected for validation. The TOC value obtained was equal to 0.48 g/mL for the rinsing technique and 3.49 mg/mL for the swab technique.

For the cleaning agent, the most restrictive criterion is the limit of 10 µg/mL of contaminant in the subsequent product, which was calculated only for the rinsing technique, as this is the method used to assess the residue of the cleaning agent only. The value obtained was equal to 3.512 g/mL, which corresponds to pH value of 9.94. To be rigorous, the same criterion used for the water for injection (WFI) was here used, which corresponds to pH values between 5.0 and 7.0 because there is no evidence that the residue of cleaning agent (NaOH), at the concentration found, will not chemically affect the vaccine.

Ovais (2010) used the following criteria to validate a cleaning procedure: therapeutic daily dose, toxicological data, the 10 µg/mL criterion and the visible residue criterion. The author then selected the method that yielded the lowest acceptance limits (i.e., the most rigorous test) and also concluded that the visible residue criterion could be used for routine monitoring purposes. Accordingly, in the present research, the most restrictive testing criterion was selected.

Estimation of the product recovery

Sampling by rinsing

Equation 6 was used for the calculation of the percent recovery. Results obtained are presented in Table 4. When sampling by the rinsing technique was selected, the percent Hib recoveries ranged from 98.5 to 100.9%. The value of 98.5% was the lowest obtained and was used to normalize the percent recovery results obtained for the rinsing technique during the validation testing.

Table 4
Average product recovery after rinsing

	Hib vaccine (water rinsing)	
Stainless steel 316 L coupon	Average concentration (µg/mL)	% recovery
Coupon 1	0.882	98.5
Coupon 2	0.898	100.9
Coupon 3	0.891	99.8
Coupon 4	0.891	99.9
Coupon 5	0.897	100.6
C_{pos} (Positive control)	0.892	98.5
C_{neg} (Negative control)	0.193	98.5

Before cleaning validation programs were instituted, visual inspection was the sole means of confirming equipment cleanliness. Forsyth *et al.* (2006a) state that the use of visual inspection as the sole criterion for cleaning validation is not advisable. The authors suggest the use of swab testing beyond the qualitative visual inspections. A similar argument is used to suggest the use of rinse sampling in conjunction with swab results, as performed in the present work.

Sampling by swab

To assess the recovery factor by swab, Equation 6 was used to calculate the values for each run. The lowest percentage of recovery calculated was used as the final result. Results from Table 5 show that the lowest percent recovery is 98.4%, which was used to normalize the results from swab sampling obtained during the validation testing. Forsyth *et al.* (2006b) concluded that visible cleanliness criteria were more rigid than quantitative calculations. Alternatively, they mentioned that the US Food and Drug Administration limited the use of the visibly clean criterion between different lots of the same products, indicating the need for a more reliable source of investigation.

Table 5
Average product recovery after swab recovery

	Hib vaccine (swab)	
316 L Stainless steel coupon	Average concentration (µg/mL)	% recovery
Quadrant 1	4.71	98.6
Quadrant 2	4.70	98.4
Quadrant 3	4.75	99.6
Quadrant 4	4.73	99.2
Quadrant 5	4.75	99.5
$C_{pos.swab}$ (Positive control)	4.72	98.6
$C_{neg.swab}$ (Negative control)	0.341	98.4
$C_{neg.plaque}$ (Negative plate control)	0.389	98.4

3.5. Estimation of the recovery of the cleaning agent

Results obtained for the recovery of the cleaning agent are presented in Table 6; the lowest percent recovery value is 98.1%. These results demonstrate that this method effectively recovered NaOH solution from 316 L stainless steel. It was not necessary to correct pH for samples of rinsing water because the values obtained were between the criteria suggested by the United States Pharmacopeia, pH = 5.0 to 7.0.

Table 6
Average product recovery for the cleaning agent

	NaOH (water rinsing)	
Stainless steel 316 L coupon	Average molar concentration	% recovery
Coupon 1	7.29×10^{-5}	99.1
Coupon 2	7.28×10^{-5}	98.9
Coupon 3	7.22×10^{-5}	98.1
Coupon 4	7.25×10^{-5}	98.5
Coupon 5	7.26×10^{-5}	98.6
C_{pos}	7.36×10^{-5}	98.1

3.6. Cleaning and sampling of the tank

All results from cleaning and sampling of the tank are shown in Table 7. The results are adjusted for the total recovery possible, as previously described.

The summary of all validation tests performed is presented in Table 7. Results indicate that for each sample collected, the specific parameters can be calculated, demonstrating that the methods used to validate the cleaning procedure after exposure to a vaccine formulation were suitable for this purpose.

From Table 7 it can be observed that bacterial endotoxin concentration found in sample A was greater than 250 EU/ML, indicating that the tank was effectively contaminated for the evaluation of chemical pyrogen removal. With the results obtained from sample B it could be observed that the cleaning procedure performed in the tank was efficient enough to reach the specified limit for product quantification, using swab technique in the bottom, sides and collector of the equipment.

Table 7
Summary of the validation results

Sample	**Results**		
	1st. Run	**2nd. Run**	**3rd. Run**
A (endotoxin)	> 250 EU/mL	> 250 EU/mL	> 250 EU/mL
Visible residues	No	No	No
B (TOC)	Rod: 0.596 µg/mL Side: 0.524 µg/mL Bottom:0.339 µg/mL	Rod: 0.215 µg/mL Side: 0.226 µg/mL Bottom: 0.229 µg/mL	Rod: 0.216 µg/mL Side: 0.468 µg/mL Bottom: 0.516 µg/mL
C_{neg} *swab* (TOC)	0.544 µg/mL	0.231 µg/mL	0.485 µg/mL
C	TOC: 0.0183 µg/mL Cond.:0.914µS/cm pH: 5.5	TOC: 0.0366 µg/mL Cond.:0.636 µS/cm pH: 5.6	TOC: 0.0995 µg/mL Cond.:0.676 µS/cm pH: 5.3
D, F, I, K, M and O (endotoxin)	< 0.250 EU/mL	< 0.250 EU/mL	< 0.250 EU/mL
E	TOC: 0.0535 µg/mL Cond.:0.670µS/cm pH: 5.6	TOC: 0.0543 µg/mL Cond.:0.648 µS/cm pH: 5.7	TOC: 0.195 µg/mL Cond.:0.611 µS/cm pH: 5.4

G (TOC)	Rod: 0.525 µg/mL Side: 0.163 µg/mL Bottom: 0.257 µg/mL	Rod: 0.133 µg/mL Side: 0.165 µg/mL Bottom: 0.229 µg/mL	Rod: 0.489 µg/mL Side: 0.530 µg/mL Bottom: 0.148 µg/mL
C_{neg} *swab* (TOC)	0.349 µg/mL	0.564 µg/mL	0.569 µg/mL
H	TOC: 0.0498 µg/mL Cond.:0.678µS/cm pH: 5.6	TOC: 0.0650 µg/mL Cond.: 0.819µS/cm pH: 5.8	TOC: 0.151 µg/mL Cond.:0.769 µS/cm pH: 5.1
J	TOC: 0.0286 µg/mL Cond.:0.797µS/cm pH: 5.6	TOC: 0.151 µg/mL Cond.:0.985 µS/cm pH: 5.7	TOC: 0.193 µg/mL Cond.:0.647 µS/cm pH: 5.7
L	TOC: 0.00843µg/mL Cond.:0.782µS/cm pH: 5.6	TOC: 0.0650 µg/mL Cond.:0.819 µS/cm pH: 5.8	TOC: 0.00406 µg/mL Cond.:0.637 µS/cm pH: 5.7
N	TOC: 0.0286 µg/mL Cond.:0.797µS/cm pH: 5.6	TOC: 0.151 µg/mL Cond.:0.985 µS/cm pH: 5.8	TOC: 0.193 µg/mL Cond.:0.647 µS/cm pH: 5.7

Sample C, obtained from the final rinsing water, also indicates the efficient nature of the cleaning, as TOC determination, pH measurement and conductivity measurement presented results inside the limits specified, clearly indicating that the final residue in the tank, as well as the cleaning agent were at acceptable levels. Accordingly, sample D, obtained from the final rinsing water showed an endotoxin bacterial concentration below the limit value, corroborating the chemical depirogenization of the tank.

According to the results reported for samples G (quantification of the product by swab technique), H (quantification of the product in the rinsing water and quantification of the cleaning agent) and I (evaluation of the endotoxin concentration), it can be seen that all the results were in acceptable limits, indicating that the time and storage conditions of the clean tank were fully satisfied.

Again, the results observed for samples L (quantification of the product in the rinsing water and quantification of the cleaning agent) and M (endotoxin concentration), indicate that the specified limits were not overtaken, indicating that the steam used for sterilization did not affect the cleaning process.

Samples E, F. J. K. N and O (Water for Injectables) were investigated in the distinct steps of the process validation. All the samples presented results inside the criteria stated by the American Phamacopeia (2010), confirming that this water for injectables can be used in the process.

According to these overall results, it can be considered that the validation is approved for manual cleaning of this type of multipurpose tank, used for Hib and A and C Meningitis vaccines.

CONCLUSIONS

This cleaning validation confirmed that it is a reliable procedure for cleaning multipurpose tanks that have been exposed to formulations of Hib and Meningitis vaccines by demonstrating that the waste product is removed and that the cleaning agent is at an acceptable level. Also, this validation may contribute to an increase in industrial production, as just one tank can be used for the formulation of more than one product. Further, this validation presented information regarding the inexistence of cross contamination.

The results obtained in this study were within the limits specified by the criteria, and therefore this cleaning validation process can be considered approved. This cleaning validation is applicable for the manual cleaning process of this kind of multipurpose tank, which is used to formulate Hib and Meningitis A and C vaccines; the methodology developed specifically for this validation may require adaptations for use in other devices and other active substances.

REFERENCES

1. American Pharmacopeia, www.usp.org, (Access on April, 2010).
2. ANVISA, *Guias relacionados à garantia de qualidade*. Brasília: ANVISA, 2006. Available at www.anvisa.gov.br (Access on October, 2010).
3. Forsyth RJ, Hartman J, van Nostrand V 2006a. Risk assesment of visible residue limits in cleaning validation. Pharm Technol Issue of September 2.
4. Forsyth RJ, Roberts J, Lukievics T, van Nostrand V 2006b. Correlation of visible-residue limits with swab results for cleaning validation. Pharm Technol Issue of November 2.
5. Leblanc DA. 2000. Validated Cleaning Technologies for Pharmaceutical Manufacturing. New York: CRC.
6. Ovais M. 2010. Statistically justifiable visible residue limits. Pharm Technol 34:58-71.
7. Pinto TJA, Kaneko TM, Ohara MT. 2003. Controle Biológico de Qualidade de Produtos Farmacêuticos, Correlatos e Cosméticos. São Paulo: Atheneu Editora.
8. Sánchez JAM. 2006. Equipment cleaning validation within a multi-product manufacturing facility. BioPharm Intl 19:1-5.
9. WHO, *WHO Expert Committee on Specifications for Pharmaceutical Preparations*. 2006. Geneva: OMS, Annex 4.