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Evaluation of the stability of measles vaccine out of the cold chain under extended controlled temperature conditions

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ABSTRACT

Measles outbreaks occur periodically in remote and difficult to reach areas in countries such as the Democratic Republic of Congo. The possibility to keep measles vaccines at temperatures outside the cold chain for a limited period prior to administration would be an advantage for organizations such as Médecins Sans Frontières, which repeatedly respond to measles outbreaks in difficult contexts.

Using stability data at 37 °C and 40 °C provided by Serum Institute of India Private Limited we applied the product release model for Extended Controlled Temperature Conditions (ECTC) to evaluate the possibility of an out of the cold chain excursion.

Measles vaccine in the lyophilized form remains above the minimum required potency at the end of the shelf-life for up to 6 days at 37 °C or for 2 days at 40 °C.

This evaluation supports the use of a monodose presentation of measles vaccine in ECTC. This could be an advantage for outbreak response in isolated and difficult to reach areas. However the operational advantages of this approach need to be established.

1. Introduction

1.1. Background

Routine immunization with measles containing vaccines combined with Supplementary Immunization Activities have contributed to a large decrease in measles incidence and mortality worldwide [1]. It is estimated that 23.2 million measles deaths were prevented during 2000–2018 [1]. However despite progress, there are still considerable challenges in reaching high levels of vaccination coverage and measles continues to be one of the leading causes of vaccine preventable death among young children [2]. It has been recognized that one of the main barriers to effective delivery of vaccines are the strict cold chain conditions which require vaccines to be kept between 2 and 8 °C at all times [3]. The maintenance of the cold chain is particularly difficult in resource-poor countries where there is lack of refrigeration equipment and power supply and where immunization activities aim to reach populations living in remote areas. Measles remains particularly difficult to prevent and control in Democratic Republic of Congo (DRC). From the start of 2019, around 310,000 suspected measles cases have been reported in DRC, leading to over 6000 deaths and becoming the world's worst measles epidemic [4]. Factors such as low vaccination coverage among vulnerable communities, difficult access by vulnerable populations to health care and insecurity have contributed to the extension of the outbreak [4]. The international non-governmental organization Médecins Sans Frontières (MSF) is repeatedly responding to measles outbreaks in DRC, vaccinating over 5 million children since 2013. These outbreak response activities are often conducted in remote and difficult to reach areas where roads are limited and in poor condition. Access to these areas can take several days by motorbike and vaccines, cold chain equipment and materials may need to be carried by hand.

Stability studies have shown that second generation measles vaccines in the lyophilized form have enhanced thermostability [5]. This has been confirmed by immunogenicity studies in Cameroon and Papua New Guinea. These studies were conducted in the late 1970s and mid-1980s and showed that measles vaccines were

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seroprotective when exposed to temperatures of around 25 °C for of up to 7 days [6,7]. However, these vaccines quickly lose their potency when reconstituted and kept at temperatures above 2–8 °C [5].

In October 2015, WHO adopted guidelines to evaluate the stability of vaccines for use in Extended Controlled Temperature Conditions (ECTC) [8]. The guidelines describe the criteria for the stability evaluation of vaccines exposed to temperatures above 8 °C for a short term immediately prior to administration [8]. Taking into consideration a practical or programmatic perspective, WHO defined the minimum programmatic requirements for vaccines to be used outside the cold chain. These are defined under the term Controlled Temperature Chain (CTC), which requires a vaccine to tolerate temperatures of at least 40 °C for a minimum of 3 days. In this way and according to WHO *"ECTC encompasses CTC but is independent of the specific programmatic requirements of the current WHO CTC programme"* [9]. Although WHO has thus far only validated vaccines complying with CTC minimum programmatic requirements (three days/40 °C), the appropriateness of this CTC designation is being constantly monitored to address new vaccines and changes in programmatic strategies [10]. This provides an opportunity for alternative out-of-cold-chain use conditions.

We considered that the use of measles vaccines out of the cold chain, under ECTC, would be an advantage for MSF outbreak response activities, particularly in contexts such as DRC where MSF repeatedly responds to measles outbreaks. In order to evaluate the appropriateness of such a use, we aimed to assess the stability of measles vaccine manufactured by Serum Institute of India Private Limited (SIIPL) using the mathematical modelling and statistical concepts developed in WHO's ECTC guideline [8]. For this, we used available stability data provided by SIIPL that had been generated for regular accelerated and long-term stability testing purposes and not specifically for ECTC testing. This assessment is a first step to contemplate the potential use of existing presentations of measles vaccines outside the cold chain, in accordance with ECTC principles. If deemed satisfactory, from regulatory and operational perspectives, this work could lead to the pursuing by the manufacturer of a formal regulatory submission to the relevant authorities with the aim of obtaining a relabelling of the vaccine that takes into account the validated excursion time and temperature conditions.

ECTC methodology has been developed by WHO and endorsed in 2015 by WHO's Expert Committee on Biological Standardization. This methodology is the only guiding document on the scientific and regulatory issues to be considered in evaluating the stability of vaccines for use under ECTC.

2. Methods

The evaluation of vaccines for use in ECTC is based on a product release model that considers potency decay rates at different storage conditions and two main thresholds, the Minimum Release Potency (MRP) and the end-of-shelf-life lower limit (LL) (Fig. 1) [8]. The MRP corresponds to the manufacturer's established minimum potency that needs to be present at batch release. The LL refers to the minimum immunizing dose or minimum potency that the vaccine should have at the end of the shelf-life. To ensure generalizability of results, the evaluation accounts for a "worst-case" scenario considering a vaccine released close to the MRP and exposed to temperatures outside 2–8 °C at the end of the shelf-life [8]. Adequate potency, this is above the established LL, should be present to account for the decay of potency during shelf-life and during the planned exposure [8].

For measles vaccine, the LL is determined at 1000 viral infective units per dose (i.e. 3.0 $Log_{10}CCID_{50}$/0.5 ml) [11]. The MRP is defined by SIILL as 3.5 $Log_{10}CCID_{50}$/0.5 ml. By using the MRP as starting point, we calculated the statistical lower bound (LB) of the mean potency of measles vaccine maintained at 2–8 °C and exposed at >8 °C at the end of the shelf-life. As measles vaccine needs to be reconstituted prior to administration, the exposure period includes potency decay rates of the lyophilized form and of the reconstituted vaccine. The calculations take into account an uncertainty variable that includes the assay precision and the standard error of the decay slope at each temperature. For a vaccine to meet specifications, the statistical LB should not exceed the LL.

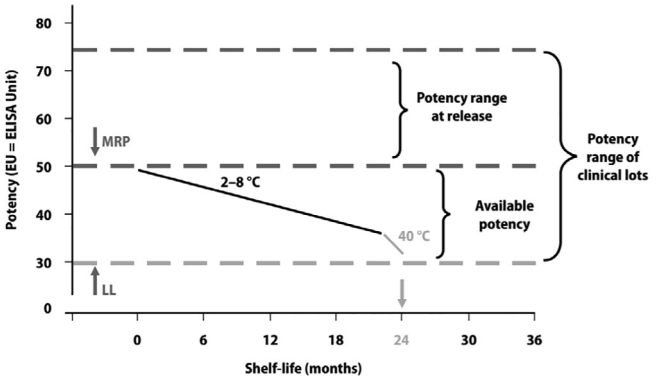

Fig. 1. Graphic representation of the product release model for ECTC evaluation (From: WHO Guidelines on stability evaluation of vaccines for use under extended controlled temperature conditions. Sixty-sixth Report; WHO Technical Report Series No. 999, 2016, Annex 5; page 243).

The LB is calculated as follows:

$$LB_{1-\alpha} = MRP + t_1 b_1 + t_2 b_2 + t_3 b_3 - U$$

where

1-α	Statistical confidence level associated with the lower bound of the 95% confidence interval (α = 0.05)
MRP	Manufacturer's minimum release potency (defined as 3.5 $Log_{10}CCID_{50}$/0.5 ml)
t_1	Time at temperature in the cold chain or shelf-life in cold chain (24 months)
b_1	Decay slope at 2–8 °C during shelf-life
t_2	Time at >8 °C lyophilized form
b_2	Decay slope >8 °C lyophilized form
t_3	Time at >8 °C reconstituted form
b_3	Decay slope >8 °C reconstituted form
U	Uncertainty associated with the independent estimation of the numbers on the right side of the equation:

$$U = z_{1-\alpha} \text{xsqrt}((S_{assay})^2 + (t_1 s(b_1))^2 + (t_2 s(b_2))^2 + (t_3 s(b_3))^2)$$

where

$z_{1-\alpha}$	The one sided z statistic at the confidence level associated with the 95% CI (α = 0.05)
S_{assay}	Assay precision (established as 0.055)
$s(b_1)$	Standard error of the decay slope during shelf-life at 2–8 °C
$s(b_2)$	Standard error of the decay slope at >8 °C lyophilized form
$s(b_3)$	Standard error of the decay slope at >8 °C reconstituted form

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Decay slops at each temperature and period were calculated using existing stability data from long-term and accelerated stability studies conducted by SIIPL on measles vaccine and the measles component of the combined measles and rubella (MR) vaccine. For the decay during shelf-life at 2–8 °C, data from the measles component of the MR vaccine from four batches was used with potency data for baseline, 3, 6, 9, 12, 18, 24 and 36 months of storage. For the decay in potency at 37 °C in the lyophilised form we used a total of 20 batches, 12 from measles vaccine and 8 from the MR vaccine, with data points at baseline, 7, 14, 21 and 30 days after exposure at 37 °C. For the decay in potency at 40 °C in the lyophilized form, we used the data of the measles component from 9 batches of MR vaccine with data points at baseline, 3, 6, 12 and 18 days of exposure at 40 °C. Similarly, for the reconstitution period, data for the measles component from 9 batches of the MR vaccine were used with hourly points from baseline to 8 h post-reconstitution. All stability data for measles vaccine and the measles component of the MR vaccine were from the 10 dose vaccine presentation and were generated by SIIPL under laboratory conditions as part of their regular stability testing plan. Data for the measles component of the MR vaccine was used to complement or to substitute, when lacking, data on measles as a single antigen. For each period, shelf-life at 2–8 °C, exposure of the lyophilized vaccine at 37 °C and 40 °C and exposure post-reconstitution at 40 °C, potency data were log-transformed (log10) and analysed by linear regression (Stata, version 14).

3. Results

The decay in potency during shelf-life was estimated at −0.003 (95%CI: −0.006 to −0.001) $Log_{10}CCID_{50}$/dose per month of storage. We estimated that at the end of the 30 months shelf-life, the product would have a remaining potency of 3.403 $Log_{10}CCID_{50}$/dose.

A decay rate of −0.072 (95%CI: −0.079 to −0.064) $Log_{10}CCID_{50}$/dose per day was estimated for the measles component of the MR vaccine when vaccine is exposed at 40 °C. No data at 40 °C were available for the measles vaccine as single antigen. If we consider only the potency data for the first 2 points (initial and 3 days), the decay slope increases to −0.139 (95%CI: −0.181 to −0.097) $Log_{10}CCID_{50}$/dose perday (Table 1). Using the most conservative estimate, the LB at 3 days exposure at 40 °C is 2.995 $Log_{10}CCID_{50}$/dose, and the 3.0 $Log_{10}CCID_{50}$/dose (LB) is reached at 2.9 days of exposure to 40 °C (Table 2).

For the reconstituted vaccine, using the data from MR vaccine, the decay slope at 40 °C was estimated at −0.108 (95%CI: −0.235 to −0.020) $Log_{10}CCID_{50}$/dose per hour (Table 1). In order to account for 1 h exposure at 40 °C post-reconstitution, the available time in the lyophilized form decreases to 2.1 days (Table 2).

Using the stability data at 37 °C, we obtain a decay slope of −0.026 (95%CI: −0.029 to −0.023) $Log_{10}CCID_{50}$/dose per day. However, similarly to the exposure to 40 °C we see a higher loss in titres at the beginning of the exposure and if we take only the 0 and 7 days data points the decay slope increases to −0.061 (95% CI: −0.073 to −0.048) $Log_{10}CCID_{50}$/dose per day (Table 1). With this decay slope, the LB 3.0 Log10CCID50/dose limit is reached after 6.6 days exposure at 37 °C.

As stability data post-reconstitution at 37 °C is not available, we applied the decay slope post-reconstitution at 40 °C to the ECTC model. The results show that to allow1 hour post-reconstitution, the available time before reconstitution decreases from 6.6 to 4.8 days (Table 2).

Table 2
Time available before reaching the LL or minimum potency.

	Before reconstitution	After reconstitution*
At 40 °C	2.9 days	Immediate administration
	2.1 days	1 h
At 37 °C	6.6 days	Immediate administration
	4.8 days	1 h

* Assuming reconstitution always at 40 °C as no data at 37 °C was available.

4. Discussion

In this study we have applied the WHO product release model to evaluate the stability of measles vaccine for use under ECTC. The results show that the measles vaccine manufactured by SIIPL can tolerate a single excursion at 40 °C for 2 days and at 37 °C for 6 days. In both cases the analysis accounted for the "worst case scenario" where a vaccine released at the minimum release potency is taken outside the cold chain at the end of the 30 months shelf life. Once reconstituted, vaccine potency decreases very quickly, allowing only 1 h storage post-reconstitution if time of excursion outside the cold chain in the lyophilized form is reduced to 4 days at 37 °C and kept at 2 days at 40 °C.

Our analysis has some limitations. These are primarily due to the use of existing stability data not specifically generated for ECTC purposes. Although we aim to evaluate the stability of measles vaccine under ECTC, we also included data for the measles component of the MR vaccine. Moreover, to account for variations on the linearity of the decay rate, we considered the stability data as close as possible to the expected excursion time-window (i.e. 0 and 3 days for 40 °C and 0 and 7 days for 37 °C). However the number of data points within the time-window are few and sometimes outside the ECTC exposure time considered, reducing the robustness of the stability estimates. This, has, however, resulted in higher decay slops and consequently a conservative estimation of the stability of the vaccine at temperatures outside the cold chain. Although the stability data was generated from 10 dose vials, we consider that the results and conclusions apply to other SIIPL measles vaccine presentations. These limitations were deemed acceptable considering that this is an initial assessment aimed to inform the appropriateness of the use of measles vaccine under ECTC, both from stability and operational perspectives.

Table 1
Potency decays used for the evaluation of CTC/ECTC.

Product form	Storage	N° Batches		Stability data time points	Decay slope	95% CI	SE
		M	MR				
Lyophilized	2–8 °C	0	4	Initial, 3, 6, 9, 12, 18, 24 & 36 months	−0.0031	(−0.0059 to −0.0009)	0.0011
Lyophilized	40 °C	0	9	Initial, 3, 6, 12 & 18 days	−0.0715	(−0.0789 to −0.0641)	0.0036
Lyophilized	40 °C	0	9	Initial & 3 days	−0.1388	(−0.1810 to −0.0966)	0.0199
Lyophilized	37 °C	12	8	Initial, 7, 14, 21 & 30 days	−0.0258	(−0.0289 to −0.0227)	0.0016
Lyophilized	37 °C	12	8	Initial & 7 days	−0.0607	(−0.0732 to −0.0482)	0.0062
Reconsituted*	40 °C	0	9	Initial, 1, 2, 3, 4, 5, 6, 7 & 8 h	−0.0884	(−0.1009 to − 0.0760)	0.0063
Reconsituted*	40 °C	0	9	Initial & 1 h	−0.1076	(−0.2353 to 0.0202)	0.0603

* Stability data post-reconstitution was available only for storage at 40 °C

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If applicable, this initial assessment could trigger interest and investment on the manufacturer's side in order to fill any data and analyses gaps necessary for a regulatory submission.

It is important to consider that measles vaccines do not contain preservatives and as such once reconstituted need to be keep between 2 and 8 °C for a maximum of 6 h to reduce the risk of microorganisms growth in case of accidental contamination [12]. Additional studies should focus on evaluating the real risk of microorganisms' growth when vaccines are left outside the cold chain. Until the safety is proven, the use of measles vaccine is limited to a single-dose presentation, which allows the immediate administration of a reconstituted vaccine. The use of monodose presentation out of the cold chain presents, however, some constraints. For instance, monodose vials of measles vaccine have approximately the same volume as 10-dose vials, requiring higher cold chain capacity at all levels of the cold chain, and costs 4 times more. Whereas the 40 °C excursion for 2 days may have limited applicability, the 6 days exposure at 37 °C can be of higher interest in particular situations. The use of monodose presentation out of the cold chain, under ECTC, could be an advantage for vaccinating remote and difficult to reach populations in countries like the DRC and could be particularly useful in outbreak response, where coverage and reactivity are key to ensure the success of the intervention. The implementation of these approaches should be associated with dedicated monitoring of the temperatures and the number of days the vaccine is exposed to temperatures outside the cold chain to ensure the stability of the vaccine remains adequate.

We are cognizant of the fact that neither of the two excursion scenarios presented here comply with the WHO CTC requirements of 3 days at 40 °C. Moreover, it is important to note that an ECTC use would require the manufacturer to compile and submit a variation dossier to the National Regulatory Authority (NRA), in this case the Indian CDSCO. Upon approval from the NRA the dossier would have to be submitted to WHO prequalification programme for relabelling approval at international level [8]. However, at this point, the lack of operational evidence of the value-added of the use of a monodose measles vaccine under ECTC/CTC might be an important deterrent for further investment. A pilot study to evaluate the monodose presentation of measles vaccine under ECTC could provide the operational answers to the conditions of its use, including the added value in reaching hard to reach populations, the use of different presentations, such as 10 dose vials for mass use and 1 dose vials for areas reached by ECTC approach, and time saved in outbreak response. Adding operational experience to the stability assessment will help informing the way forward, including discussions with global partners involved in immunisation such as WHO, Gavi and UNICEF.

5. Conclusion

Although there has been significant decrease in measles cases around the world, measles epidemics continue to occur highlighting immunity gaps and deficiencies in current preventive activities. In places such as DRC, rated as one of the countries with the highest number of unvaccinated children worldwide [13], the geographical accessibility, sometimes in conjunction with insecurity, have been recognised as causes of low vaccination coverage and worrying epidemics [14]. Insufficient functional cold chain coverage has also been identified as one of the factors contributing to low immunization coverage in some provinces of the DRC [14]. In this situation, innovative strategies are needed in order to reach the unvaccinated and undervaccinated and achieve the immunity threshold required to eliminate measles.

This study represents an effort to characterise the stability of measles vaccine to evaluate the potential use under ECTC approach. The results of this initial assessment of the stability of measles vaccine outside the cold chain using the statistical methodology of WHO's ECTC guideline reveal that vaccine's potency remains above the minimum threshold for 6 days at 37 °C and 2 days at 40 °C. These results provide the possibility for an ECTC use of measles vaccine as a monodose presentation. However, the operational added value of the ECTC approach with the measles vaccine needs to be established. The constraints related to the monodose presentation, together with the need for careful temperature and time monitoring and the handling of a mixed supply, with single dose vaccine under ECTC and multi-dose vials in cold chain should be explored and documented before moving forward.

Pilot implementation studies in the field will help explore the programmatic feasibility and determine optimal use conditions for the monodose presentation of measles vaccine.

CRediT authorship contribution statement

Aitana Juan-Giner: Methodology, Formal analysis, Writing - original draft, Writing - review & editing. **Alain Alsalhani:** Methodology, Writing - original draft, Writing - review & editing. **Isabella Panunzi:** Conceptualization, Writing - review & editing. **Vincent Lambert:** Conceptualization. **Michel Van Herp:** Conceptualization, Writing - review & editing. **Sunil Gairola:** Methodology, Resources, Writing - review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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