In Search of Better Anemia Estimates: USAID Advancing Nutrition's HEmoglobin Measurement Project

Webinar Transcript

Lauren Wheeler

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Okay, great. So we will go ahead and get started. Thank you so much for joining today's webinar on USAID Advancing Nutrition's HEmoglobin MEasurement project. My name is Lauren Wheeler and I am a Project Coordinator with USAID Advancing Nutrition. I will kick us off with some Zoom housekeeping and reminders that you may find useful during the webinar. Next slide, Emily.

If at any point during today's webinar, you are unable to hear the speakers, please make sure you have connected your audio by selecting the headphones icon at the bottom of your Zoom window. Please send a chat message to everyone to introduce yourself, send in your comments or questions, or ask for tech support during today's session. Closed captioning in English has been enabled for this meeting.

To view the live English subtitles on your screen, click on this CC icon and select to show subtitle. Finally, please note that this meeting is being recorded and live-streamed. Next slide. While the webinar is in progress, please submit your questions for the panelists in the Q&A box. Panelists will either reply back to you via text in the box or will answer your questions during the Q&A discussion portion of the webinar. I will now pass it to our moderator, Silvia Alayón. Over to you. Silvia.

Silvia Alayón

Thank you so much, Lauren. Good morning, good afternoon, or evening, everyone. Welcome to today's webinar, In Search of Better Anemia Estimates, where you'll learn about USAID Advancing Nutrition's HEmoglobin MEasurement project. We're so grateful that you could join us today and are very excited to share with you preliminary results from several years of work on the topic.

My name is Silvia Alayón. I'm the Director for Measurement on USAID Advancing Nutrition and I will be your moderator for today's webinar. As an overview of our agenda today, we'll kick off with some opening remarks from our donor, USAID, to whom we are grateful for their funding and continued technical involvement in this work. This will be followed by a presentation describing our six-country study to assess the accuracy and precision of hemoglobin measures obtained using various HemoCue

models and three different types of blood samples, venous, pooled capillary, and single drop capillary blood.

During that presentation, we will be providing you with a glimpse of some of the results which we're still in the process of preparing for publication, so keep an eye out. That will be out later in the year, we hope. We're extremely lucky also that we are joined today by the researchers from five of the six study sites. Following the two presentations, we will have a panel discussion with the researchers from these sites who have generously agreed to join us to share their experiences, challenges, and the many lessons learned throughout the process. I think you might also hear of some pending questions that they have after all of their work. Next slide please, Lauren.

Our first speaker today is Dr. Omar Dary, a Senior Nutrition Science Specialist at the Bureau for Global Health at USAID, Washington. Since 1990, he has worked in public health nutrition from basic research to strategic planning at national and global levels and has provided technical assistance to over 45 countries. His major areas of expertise are micronutrient interventions, especially food fortification, and assessment of micronutrient status. Omar has a bachelor's in biology with an emphasis on analytical chemistry and biochemistry from San Carlos University in Guatemala. He holds a PhD in Biochemistry from the University of California, Riverside. Welcome, Dr. Dary, and over to you.

Dr. Omar Dary

Thank you, Silvia. Good morning and good afternoon, colleagues. I'm impressed, like the audience which is coming from all the continents, and thank you for accepting this invitation. May I have the first slide? The prevention and reduction of anemia. Next slide, please. The following one is one of the World Health Assembly targets. In the year 2012, it was proposed and it was accepted by the countries to achieve 50% reduction of anemia in women of reproductive edge. That's the figures of 2012.

There have been some discussions to extend the end date of this target for the year 2030 to coincide with the UN as developing world system. We can ask ourselves is this something new? A few of us, we have been in this area for a long time. We can learn the following. You can press the next slide. The following one. In the year 1990 when we wanted to reduce the deficiencies of iodine, vitamin A, and iron, it was established that the reduction of iron deficiency anemia in women by one-third in the year 1990, and nothing happens. It could be the same story now that we have a new target that was established in 2012. That is why it's so important that we understand what anemia is.

In USAID, we promoted through our project, USAID Advancing Nutrition, the establishment of an advisory group that we call the Anemia Task Force and they have been discussing what is the nature, the assessment, and the prevention and the interventions of anemia. In today's webinar, we are going to be in a very specific area, how to measure anemia correctly and why is that it important. May I have the next slide?

This is a very good paper that WHO colleagues with other partners they published last year. They took the work of reviewing all the information that was able and available for the last 20 years about the performance and the evolution of anemia in the different countries and in the different continents on the Earth. If you go and look at that paper, they have an annex that you can have all the information country per country.

In this occasion, I only presented the very high level at the different continents and the global and the blue lines thus are the total anemia. Then you have the other colors, the mild anemia, the moderate anemia, and the severe anemia, and we focus only in the total anemia. It is striking to see that the reduction of the prevalence of anemia in all the different continents has been very stable during the last 20 years.

Some of the continents have some reduction like South Africa or Latin America, but that is not 50% reduction in 20 years. Then that means that we need really to study what was our target, what is the

reasons of this not change anemia prevalence? We need to look at anemia as an ecological adjustment of the human being to the environment. Our mistake has been related anemia only to iron deficiency, and that is what in the 1990s, was called iron deficiency anemia. That error has continued so far.

We need to understand that because anemia is a very complicated indicator, a very high leveling bio-indicator that we need to find the genetics, the reactions to the environment, how the people and the bodies react to the infections. We have learned in the last 20 years that the absorption of iron does not depend only in the quality of iron in the diet, depends a lot in the metabolism of the host. If the host is infected or inflamed, and that includes overweight and obesity, the body is going to stop the absorption and the mobilization of iron and that is going to be seen as iron deficiency. It's not iron deficiency by intake, it's iron deficiency that we call functional iron deficiency.

All of these things are influence the anemia, but nowadays go back to-- if we are measuring hemoglobin concentration well, in order to follow that target of the World Health Assembly. You press again for the next slide. We see these results. Some of the questions that we can ask ourselves. Where the hemoglobinopathies of the different countries taken in consideration for making adjustments in the determination of the anemia prevalence?

Because we have been as a species for thousands of year trying to survive in infected environments. One of the most important diseases is malaria. Having something like a lower hemoglobin concentration will help us to survive. That mean that the hemoglobin concentration is an equilibrium to keeping alive and to freely work the physiological functions. Do the geographic region that I presented in this slide have the same environmental and health conditions? They are not the same, which means in order for all the countries to imitate what is happening in the high-income countries, we need to change all the conditions, economical, environmental, the diets, the health status and access that are similar, like exist right now in the high-income countries and probably that will take more than 20 years.

What about the methods to determine hemoglobin in each of these regions? Some of the regions probably use venous blood and hemoglobin auto-analyzers. Others the very popular insert use of different machines, using finger-pricking blood and what it is important, I want to show you in the next slide and what this trigger all the work that we have done with this group of researchers. The next one.

Here you can see only a simple collection of the prevalence of anemia in two different types of population surveys. The famous and traditional DHSs and Micronutrient surveys. When the surveys were done in the same country in similar years, you can see the large difference of the prevalence that could be as large as 1.5 to 2.7 times. That moves the severity of the change of the prevalence of anemia in the country from severe to moderate or moderate to mild. This is important because we need really to know where is the problem of anemia in which communities in each country.

The reason of these changes, you can see that in the two types of population surveys, they were using different devices. HemoCue 201+ mostly in the DHS, and HemoCue 301 mostly in the Micronutrient surveys. They were using also different types of blood samples. In the DHSs where capillary drop by finger-pricking, most in the Micronutrient surveys was either venous blood, only a few of them pulls of capillary blood. The other problem is that every country could have different ways to have and to measure the weight. The scales are different. Then we have here something that we need to look. The next slide.

I will advance for you what are the main results that our colleagues are going to share today and that is mostly the problem of the variation of the precision of the method depending of the sample of the blood more than in the machines is the sample that is making the trouble. If we're measuring venous blood in a hematology analyzer, we have a precision about 3 grams of hemoglobin per liter.

If the same sample is analyzed in a HemoCue, that could be from 6 to 10 grams per liter. If it's a finger-prinker blood instead of venous blood, the variation could be from 15, 20, or 30 grams per liter. When

you increase the variation, then the error in the identification of anemia in an individual could be from non-dynamic to moderate anemia, only dependent in the way that the method have the variation.

If we extrapolate these to populations, it explains why we are having very large prevalence of anemia using finger-pricking blood and the HemoCues [unintelligible 00:14:57]. I end it here and now I welcome our colleagues that did this research for them to share their own experience. Thank you very much.

Silvia Alayón

Thank you so much, Omar. Now, before I introduce the next speaker, I just want to remind everyone that if you have any questions, please use the Q&A button at the bottom of your screen to submit the questions. We have many people prepared to respond in real-time. We won't have a formal Q&A session, but we'll try and address all of the questions that arise during the webinar in writing through the Q&A function.

Now, I'd like to introduce our next speaker. Our next speaker is Dr. Laura Hackl. Dr. Hackl is a consultant for USAID Advancing Nutrition and was previously a research advisor on the project, where she designed research studies and supported activities in the areas of food fortification, anemia, micronutrients, and diet quality, mostly in Sub-Saharan Africa. During that time, she also led the HEmoglobin MEasurement project.

Dr. Hackl has a PhD in Health Sciences and Technology from ETH Zurich and completed her postdoc at Cornell University. I'm really very happy that we have Laura with us today, and I'll turn it over to her to describe the study and give you a little bit a glimpse of the results. Over to you, Laura.

Dr. Laura Hackl

Thank you so much, Silvia. Thank you for inviting me to present this really, really interesting study. It was such an exciting project. If you could advance to the next slide, please, and the next one, please. Dr. Dary has already set the stage very nicely, and he already pointed out some of the issues that we face when we measure hemoglobin. If you can proceed, one.

Basically, the way to measure hemoglobin can be done with automated hematology analyzers. These are really the reference methods that I usually use with venous blood. However, those automated analyzers are not always practical, so in certain settings, they are just not available. There are portable devices such as the HemoCue, which is the most commonly used one. HemoCue currently exists in three different forms. There's the HemoCue 201+, 301, and 801. They all operate based on slightly different principles. I won't go into detail, but you will hear about the three different devices later on.

Those portable devices, those HemoCues are usually used with capillary blood samples This can either be a single drop of capillary blood from a finger prick or also a heel prick, or it can be a pool of capillary blood, so basically a collection of several drops of blood. Next, please.

As Dr. Dary already pointed out, there are various factors at different stages of the blood collection process that can affect the measurement. One is the venous or capillary blood collection, another issue is also the measurement device and other conditions such as the sample storage or analytical components can also affect the measurement, as well as environmental factors such as temperature and humidity. Next slide, please.

These issues they are not new, they've been known for some time and this is why the HEmoglobin MEasurement project came into life. Basically, as you can see on this timeline here, it all started in 2016 under one of the predecessors of USAID Advancing Nutrition called SPRING, which is where in 2016 is when the team group was established and had the first meeting. The group worked on a protocol that was then set out for public comment to address some of the issues that I mentioned earlier. Based on this protocol, there were grants awarded to address the questions, and if you go to the next slide, I will show you those questions. Next slide, please.

Basically, in those hemoglobin measurement grants that were given out, the objective was to identify the best procedures or methods to determine hemoglobin concentration or anemia prevalence in population-based surveys. Specifically for this Phase 1, the goal was to assess the performance of the three different HemoCue models, so 201+, 301 and 801 as mentioned earlier, and compare their performance to a certified hemoglobin auto-analyzer. Those, as mentioned before, are the gold standards for hemoglobin measurement, and also to understand how venous pooled capillary or single drop capillary blood samples would perform. Next, please.

The study was implemented in six different countries, so this was the very exciting part about this project because we were very happy to select grantees from very different geographies and the collaborators were from American University of Beirut in Lebanon, eHealth Africa, in Nigeria, The Instituto de Nutrición de Centro América y Panamá in Guatemala, the National Institute of Medical Research in Mwanza, Tanzania, University of British Columbia in Canada, in collaboration with HKI, Cambodia, as well as the Haramaya University in Ethiopia. We are very happy to have some of the collaborators here today and they will tell you more about their experiences later on.

Basically, the study participants in each of the studies, so each of the different sites, they followed the same protocol. I will go into more detail on what this protocol was a little bit later. Importantly, the study participants were women of reproductive age, as well as children 12-59 months of age in low and middle-income countries. Next slide, please.

To come back to the HEME protocol and the actual questions that we were asking, the two main questions that were addressed by those different study sites was about the accuracy and the precision of the three HemoCue device models. Importantly, this was all done in a controlled laboratory setting, and how the accuracy and precision compares to a certified auto-analyzer when measured in venous blood samples. The second question was, what is the accuracy and precision of the hemoglobin concentration when using venous pool capillary or single drop capillary blood, also analyzed in those three different HemoCue devices and against a certified hematology analyzer? Next slide, please.

I will walk you through this slide. This is very important to understand how this study was set up. You can see here, each study site had up to four cohorts. Why up to four? That is basically because of the HemoCue 801, which you can see in orange. That was not available in each of the study sites. We tried our best to have this device available, but only four out of six study sites were able to test this device.

You can see in the different rows there are different cohorts. Cohort 1 is in the first row and cohort 4 in the fourth row and so forth. In the columns, you can see the different blood sample types, so venous pooled capillary and capillary third drop blood. In cohort 1, and again, each cohort consisted of women of reproductive age and children 59-month age. In cohort 1, we collected venous blood and pooled capillary blood from the same individual. The venous blood was analyzed on an auto-analyzer, the gold standard, and on the three different HemoCue devices. The pooled capillary blood was also analyzed on the HemoCue devices.

Cohorts 2 to 4 are similar in that they compare venous blood both on a HemoCue device and an auto-analyzer, as well as capillary third drop blood on an auto-analyzer. Each of the cohorts tested a different HemoCue device. The reason for this setup was that we wanted to keep the burden on the participant as low as possible, so we did not want every participant to provide every blood sample. Next slide, please.

In terms of data analysis, we presented the difference in the hemoglobin concentration between the HemoCue versus auto-analyzer and determined accuracy and precision. For accuracy, we basically compare the difference from the HemoCue to the auto-analyzer by the difference from the zero line and the precision, we determine by establishing the 95% limits of agreement. If you go on the next slide, please. The precision is particularly important because we determine anemia prevalence through the

proportion of samples below the hemoglobin threshold, which is why precision is very important. Next, please.

Then as a second step, we try to improve the accuracy. Basically, we want to reduce the systematic error by adjusting for the machine bias. We did this by adjusting the HemoCue values with the regression calibration and also did adjustment via Bland-Altman, but this data we will not present here. Next, please. In terms of precision, as mentioned earlier, this is based on random error or dispersion, and therefore we cannot correct because we cannot adjust for this error. Next, please.

Here I'll just present you some results from one of the country sites, Tanzania. These are results from women and children combined from the HemoCue 201+ device. You can see in the two rows, you can see the unadjusted and adjusted values, and in the columns, you see the three different blood sample types, and what you can see here if you look at the venous column, you can see that the dispersion is quite small, so all the values cluster on the same axis. Adjustment does improve somewhat, but it's already at a pretty good stage. Then for the pool and single drop, you can see that the dispersion is relatively high and it's way larger than with the venous blood even with adjustment. Next slide, please.

When we look at the HemoCue 301 device, also in the Tanzania setting, we can see that the accuracy without adjusting is worse than compared to the HemoCue 201+. However the dispersion or precision with the pool and single drop capillary blood performs slightly better compared to venous blood as opposed to the 201+. Next slide, please.

Here you can see the accuracy of the different devices in the different countries in different study sites, and you can see that the unadjusted values in the middle column, the differences vary a lot, but you can adjust and then the absolute difference to the reference standard becomes lower. If you can go on the next, please.

Basically, with adjustment, you can improve the performance of the different devices and basically improve the accuracy to below one grams per liter when you use venous blood. Next slide, please. In terms of this version or precision, if you click on the next please, and again. You can see that venous blood has the least dispersion across the different study sites, which we could also see in the Tanzania example, and this shows that training for blood collection and both instrument use is very important, and for the capillary blood, you can see that the dispersion is quite high in across all the different sites.

To conclude, if you go on the last side, we saw that the systematic error is both device and model specific and is also the main source of error. That is basically, the blood sampling technique is the major issue that we have. To move forward, what we suggest is to basically verify each machine and determine a regression calibration adjustment to adjust for the machine bias.

In terms of the HemoCue, if systematic adjustment is done, basically any of the models can be used, whereas if no adjustment is done, the HemoCue 201+ is the device of choice. In terms of blood sample, and this is what we saw throughout the whole different sites, venous blood samples are the most precise and accurate and more accurate than pooled and single-drop capillary samples.

What we cannot emphasize enough is that the rigor in the sample collection and the training to perform the collections, et cetera, is very important since this is something that we are not able to adjust for. Before implementing, it really needs to be ensured that the personnel and everyone who is involved in the sample analysis and collection is properly trained. With this, I want to thank again everyone and hand it over to Silvia.

Silvia Alayón

Thank you so much, Laura, for that wonderful presentation and for all your hard work on this study. Now I want to shift to the next portion of the webinar and briefly introduce our illustrious panel of researchers who made all of this possible. I will introduce all five. We have five of the six countries

joining us today. I would like to introduce all of them one at a time, and then pose questions to each of them. I want to remind our panelists to please keep your cameras off until we get to your question just to avoid bandwidth issues of having too many cameras on. From Guatemala, we have Dr. Dora Inés Mazariegos, who is a researcher in the Department of Nutrition and Micronutrients at the Institute of Nutrition of Central America and Panama, INCAP. Dr. Mazariegos is a biochemist with a master's of science and human nutrition and a PhD in molecular and cellular biology. She has over 15 years of experience analyzing and interpreting biomarker data.

From Lebanon, We have Layal Jaafar who is a PhD student in biomedical sciences at the American University of Beirut. Ms. Jaafar is has a master's degree in human nutrition, and her experience includes working as a clinical dietician, research assistant, and consultant. From Tanzania, we have Dr. Kadolu Jeremiah, a clinical research scientist at the National Institute for Medical Research at Mwanza Center. He has over 15 years of experience as a researcher and consultant in nutrition interventions and infectious diseases. From Nigeria, we welcome Dr. Nirmal Ravi. He is a physician scientist and healthcare executive, currently serving as the Chief Innovation Officer at eHealth Africa Clinics and a director at eHealth Africa, where he directs a portfolio of digital health, laboratory diagnostics and medical research.

Finally, joining us from Ethiopia, we have Dr. Desalegn Ayana. He is a senior researcher and academician in infectious diseases at the University of Haramaya. He has a long history as an investigator in the areas of molecular biology with a focus on HBV, HIV, and MTB, among others. Welcome to all of you. I will direct my first question to you Dr. Mazariegos. In Guatemala, you used several HemoCue machines for each model, so you had more than one 201 more than one 301. Can you tell us a little bit about the quality control measures that you put in place during this study to account for that?

Dr. Dora Inés Mazariegos

Thank you very much, Silvia. Good morning or afternoon to all of you. I thank Advancing Nutrition for the opportunity to share some of this experience with you this day. We are talking here about reliable measurements of hemoglobin to estimate anemia. I would like to go back and say that methods for hemoglobin are very ancient. They date from end of 19th century, and they are available in the more sophisticated technologies or the simplest methods. We have revisited this theme in the last decade or so, because evidence was gathered that the easiest methodology recommended that is obtaining a drop of capillary blood by finger pricking and using portable HemoCues is prone to many errors in estimation of anemia prevalence. In my background of biochemistry and laboratory, I was also demanded by INCAP to work in the training and supervision in field operations of surveillance and systems and nutrition surveys.

I approach this team with quality controls and verification and training. This is how we approach the search. My first approach to these HemoCues started around 2008 when a critical time for Guatemala, since two different surveys had as Dr. **[unintelligible 00:38:07]** mentioned, more than 20% points of difference between the two estimates with a higher estimation of anemia with the capillary drop method. We have performed verification of the HemoCue devices training and support for field technicians. With this combination of qualified train and supervised staff along with HemoCue verification and field quality, we could ensure the reliability of the results. Since then, we have been trying to dissect the main factors causing the errors in hemoglobin measurements. We worked in 2018 in Honduras to that purpose, and we were very happy to work with the five research groups to systematically work in this purpose.

In Guatemala what we did was work with around four or three particular devices of each HemoCue model. I want leave you with the main messages this study has provided us. First variability, this is in precision is the most probable cause for error in estimating anemia with capillary drop. That is what we saw when training the technicians. Usually, first repeated drop measurements could have more to 20

grams per liter of difference. The results with qualified and well-trained staff, the HEME project demonstrated that variability increased twice or more when capillary blood was measured compared to venous blood.

That was possible, because we knew we could achieve a precision by training. The **[unintelligible 00:40:15]** results of variability of venous and capillary and single drop samples in our subjects allow us to recommend thresholds of precision that can be attainable with proper training and those minimizing random error. It appears that capillary drop variability cannot be reduced even in qualified staff hands. That is why we recommend venous blood. Also when preparing field work requiring multiple devices of the same model of HemoCue for the team surveys, we also observed that different devices had different values for liquid controls.

We also have polished results of different researchers comparing HemoCue versus clinical analyzers, and sometimes they find contradicting results for the same model. Some underestimating and some overestimating anemia.

Silvia Alayón

Exactly. Dora, can we start to wrap up? I want to make sure that we get to the other questions. Thank you.

Dr. Dora Inés Mazariegos

Thank you. We measure in different machines and we find that bias, that is systematic error, was device dependent. When correcting each machine with its own bias, we find that the results were compatible as Laura showed as. HemoCue can present systematic error, but the bias can be corrected by pre or post adjustment. We have more details of this particular Guatemalan results in the micronutrient forum, and I would like to thank this opportunity to share with you this data.

Silvia Alayón

Thank you so much, Dora. We were so lucky to have INCAP and you join us in this project, and I know we all have learned so much from you. Now, I'd like to direct a question to Layal. Can you reflect on some of the issues related to collecting pooled capillary blood samples? Feel free to turn your camera on.

Layal Jaafar

Good afternoon. Thank you for the kind introduction and for the invitation. As you pointed out, Silvia, we have challenges during the collection of the pooled capillary, and this challenges resulted in considerable variability and outliers in the pooled capillary blood results in both women of reproductive age and the children who were involved in our side in Lebanon. We believe that these variations are due to the drawbacks and the process of the pooled capillary blood collections, especially that at our side, the time for the pooled capillary blood collection was exceeding the recommended period, which is around one minute.

In some cases, and with some participants, and especially children, it was taking around two to three minutes to collect the pooled capillary, which increased the exposure of the blood to the air, and hence the oxidation, as well as the risk of coagulation of the blood. We believe that these factors affected the accuracy and the reliability of the haemoglobin measurement in the collected the pooled samples. Additionally, the process of the finger squeezing that we usually apply during the collection of the samples was painful and unpleasant, especially for the children between the 1 and the 2 years.

This discomfort didn't only complicated the process of the pooled capillary, but also prolonged the time required to achieve the required volume of the pooled capillary blood sample, which also increased the risk of blood coagulation. To effectively address these challenges that we have encountered at our side,

we propose to implement some modifications or to pinpoint some points that could be followed by those who want to collect a pooled capillary blood sample and to get an accurate results. The first point that I want to highlight is the training of the phlebotomist who are responsible for the collection of the pooled capillary blood sample.

They should be trained enough to be able to connect the pooled capillary within the recommended time and to reduce the risk of blood coagulation as well as the oxidation and we also recommend the use of alternative collection devices or techniques that could minimize the risk of blood coagulation and ensure consistent blood flow and in the case of the pooled capillary, however, this require further studies to reach this state.

To wrap up and in conclusion, we believe that the implementation of this point will significantly enhance the accuracy and the reliability of the hemoglobin measurement in the pooled capillary blood samples and this would open the door for the use of the pooled capillary as a substitute of veins blood samples in the field **[unintelligible 00:45:55]**. However, we look forward to explore more the implementation of these points or modification, and I would like to thank you for your attention.

Silvia Alayón

Thank you so much, Layal. I know we are very excited to see what else the American University of Beirut does in terms of kind perfecting the blood capillary collection. I'm going to step a little bit out of order on the questions and I want to post the next question to Dr. Desalegn, because I think you also had some thoughts to share about the collection of pooled capillary blood, especially from children and also maybe some other factors affecting hemoglobin measurement there.

Dr. Desalegn, do you want to say, add to what Layal was saying about that?

Dr. Desalegn Ayana

Okay. Thank you very much. Hello, everyone. Just to add a few points to what has been mentioned previously as a challenge. We, of course, had a procurement challenge from the beginning. We didn't have 801 HemoCue machine in Ethiopia, and also we don't have the quality control machine solution for all the HemoCue machines. It wasn't available on the market. I would like to thank one of the team members, Dr. **[unintelligible 00:47:30]**, who carry some of the materials required for this research.

We have began the challenge by procurement. In order to address one of the important points on the data collection, we actually have limited accredited autoanalyzer machines in Ethiopia. Few years ago, there we only three machines who are accredited by the National Institute, so we travel to Adama which is around 450 kilometers and the weather is a bit hot. Probably climate has also its own role. We collected the data in Adama referral and teaching hospital, which is nearly 100 kilometers away from the capital. During the data collections, obviously the data collectors doesn't have enough experience in collecting pooled capillary. We have given two days training in order to solve that, but despite that, the squeezing- mixing of tissue fluids during sample collection is one of the problems that we repeatedly faced, even though we tried to, of course, reject and try to reduce the problem.

It still persisted, because maybe the challenge is the study subjects are **[unintelligible 00:49:05]** children probably very early, because due to the pain and also the amount of sample that we are planning to collect, it finally affected the quality of the pooled capillary result.

The other challenge is the lancet type. Here in Ethiopia, we use whatever is available and we most of the time use low flow lancets, and probably high flow lancets may not be available on market most of the time. We used low flow lancets for majority of the cases and probably low flow lancets for few. Maybe the flow also has affected- maybe the type of the lancet used has affected the flow, and then therefore the measurement has been also affected in pooled capillary.

The other important things that we noticed that the challenge is that size of the test tube. We don't have pediatric test tubes with EDTA, probably 1.3 or 1.2ml EDTA tubes. We all used about the 4ml EDTA tube in order to collect the pooled capillary and also the venous capillary. These are some of the factors, including the temperature and the humidity in Adama, these are some of the factors that led to the variation that were observed on the finding. In spite all this, we have used three of the machines and then we compared it with the autoanalyzers. Thank you very much.

Silvia Alayón

Thank you so much, Desalegn, for sharing that. I think keeping in that same vein, you brought up some of the environmental factors that you encountered in Ethiopia. I'd like to pose a question about that to Dr. Kidola from Tanzania. Can you tell us a little bit about the preanalytic and environmental factors that affected the hemoglobin measurement in Tanzania?

Dr. Kidola Jeremiah

I thank you, everybody, and good morning and good afternoon. We in Tanzania we would love to share at least two-

Silvia Alayón

Excuse me, Kidola.

Dr. Kidola Jeremiah

-practical challenges that we thought were of challenge to us.

Silvia Alayón

Can you get the mic closer? Thank you.

Dr. Kidola Jeremiah

For us here in Tanzania, we would like to share at least two practical challenges that we thought could be of benefit to this forum. The first challenge that we observed in our site was environmental temperature. During implementation of our study, we observed that the high or hot environments which may be probably up to **[unintelligible 00:52:16]** or above that, our HemoCue machine was producing frequent reading error during running test. At first, we thought that we were observing was probably due to machine defect and we decided to change the machine, but the machine was not a solution.

One of our technician advised us that probably we should run the test under controlled room temperature using air condition. It is at this room-controlled temperature where you observe that our HemoCue machine was running and providing reading that were expected. It is from this lesson we learned that probably in our setting where there is very hot surrounding environment, the HemoCue machine **[unintelligible 00:52:59]** could not execute the expected hemoglobin estimate and to correct this error we need to work under a surrounding lab environment temperature probably less than 30 centigrade that our HemoCue machine be able to run without a challenge.

Another challenge that we observed as one of the practical preanalytical errors was to draw blood finger pricks, especially for women of reproductive age. During our conduct, we realized that of those who have been doing multiple pricks in order to get enough blood flow, some reported being engaged in subsistence farming like managing their farms, reporting to do raising livestock, probably cooking using firewood, or sometime they're using hand hoes for their daily activities. This participant from our setting actually had difficulty in drawing enough blood and we had to make multiple pricks to at least draw enough blood to fill the cuvette, even with non-dominant hand or even using the proparacaine side. We

thought the difficulty was due probably to their activities, because of the **[unintelligible 00:54:08]** that they had. Their approach for this was then to reorganize our team and obtain enough information on their social economic status and the activities for this to help us the team to choose the best **[unintelligible 00:54:24]** site and choose the best **[unintelligible 00:54:26]** or at least increase or maximize enough blood that could be able to run on the HemoCue machine and at least to avoid the micro fingerprints, although even this sometimes was most successful.

This two lesson meant that by the time we are learning our surveys, especially in our environments, we should be very careful with the temperature. Also, we should be careful with the number of participants we are dealing based on the activities. That are the critical practical issues that we think from our site we should like to share with the audience. Thank you.

Silvia Alayón

Thank you so much, Dr. Kidola. Now I'd like to shift the question that I will post to Dr. Ravi. I'll ask you a different question going in a different vein. Can you tell us a little bit about the challenges that you experienced in Nigeria in identifying healthy children since this was a clinic-based study? Can you talk a little bit about the enrollment and recruitment of those children?

Dr. Nirmal Ravi

Thank you, Silvia. Our study in Nigeria was done in a primary care clinic not an academic center. In Nigeria, it's similar to several African countries, I assume, where healthy children are generally not brought to the clinic for what we would normally call a well-child exam. Because of the stringent exclusion criteria in patients who had fever within the past week, any other acute illnesses, hemoglobinopathies, it was quite challenging to recruit apparently healthy children for the study.

Even though the sample size was quite small and manageable, we continued to experience problems. We really had to mobilize the community and spread the word to really recruit the required sample size, especially among children. In women of reproductive age, it was not so much of a challenge, but children we had that as a challenge. We also had the same challenge that Layal was mentioning about the capillary blood. Children under two, they're screaming and crying while we try to get the capillary pooled blood. Thank you.

Silvia Alayón

Thank you so much, Dr. Ravi. Now I'd like to hand it over for some closing remarks from Dr. Denish Moorthy, who is a senior technical advisor on USAID Advancing Nutrition. Dr. Moorthy is a physician methodologist with over 15 years of experience in global health and nutrition. Currently serving as a senior technical advisor at USAID Advancing Nutrition. Before I hand it over to Dennis, I would not be able to do that without thanking our presenters, Dr. Dari, Dr. Hackle, and all of our panelists who made this work possible. Now, over to you, Denish.

Dr. Denish Moorthy

Thank you, Silvia. Next slide, please. Now, I want to talk about, you've heard about HEME and as we are presenting the HEME results, which will be presented at the micronutrient **[unintelligible 00:57:46]** as well as in a publication soon to come to you. We are already thinking about what's next, and we've moved ahead with funding some of the studies that came with results from the first phase of HEME Grants. Next slide phase.

What issues identified as you heard, were mainly that led us to confirm that venous blood is the sample of choice, but pooled capillary blood may hold some promise as long as we can reduce the heterogeneity of the results, reduce the variation, and the I precision that we see with pooled capillary blood. Some of the pooled pre-analytical factors that we thought we should study in the short term that

we have in advancing nutrition, which is in its last year of implementation is looking at blood collection procedures and environmental factors.

At the same time as Eric Boy noted in his comments if you're collecting venous blood, there has to be issues that might reflect a venous blood collection in addition to things like cost-effectiveness and storage and transport, and also the stability of biomarkers. There's a lot of research that has already been done and we thought we would build upon some of that research with short laboratory-based studies. Next slide, please. We had 4 of the 6 original participants who are still going to carry on these stage 10 studies.

Silvia Alayón

We have one minute.

Dr. Denish Moorthy

Yes. We are studying the same group in women of reproductive age and children under 5 from age of 12 to 5. We'll have results for this by September prior to the micronutrient forum. Next slide. In looking at the various factors we are studying, you can see across these three, across the four different grantees of the second phase of the HEME studies.

We're studying things like the micro cue loading of the pooled, capillary blood impact of temperature and time on hemoglobin and pooled capillary, as well as in venous blood issues related to the volume of anticoagulant lancet type in terms of reading, how long it takes to read the results on a HemoCue. These are the pre-analytical factors that are being studied that are potentially could answer some of the questions on what leads to the variation in the pooled capillary blood that we are seeing, in spite of following a standardized protocol. In these studies, these results will be coming to you later this year in September or October. We hope to share that with you then. Before that, we'll have the HEME results published the first phase of the HEME results. I do want to make a note of all of the people since 2016 who have worked on this with the SPRING project and USAID Advancing Nutrition. It would not be possible without all of these names. I want to point out that in the list of team grantees, I mentioned the primary investigators, but they are supported by a team of investigators and research personnel who also deserve our thanks and our appreciation for the work that they do.

Finally, last but not the least to USAID for funding this work over the past few years, and hope to see their continued involvement in hemoglobin research and hemoglobin studies leading to better recommendations. Next slide, please.

We want to thank you for joining. We have the next webinar that'll be held in a couple of weeks' time on May 31st. This will be on understanding Infant and Young Child Feeding Measurement. We also have a link to a feedback survey that we would appreciate if you would fill in based on what you heard on the webinar today. With that, I would like to end this webinar and thank you all for coming and listening to us.

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