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Considerations in Using US-Based Laboratory Toxicity Tables to Evaluate Laboratory Toxicities Among Healthy Malawian and Ugandan Infants

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Abstract

Objectives—To determine normal hematologic and selected blood chemistry values among healthy, full-term, non–HIV-exposed infants in Uganda and Malawi, and to determine the proportion of healthy babies with an apparent laboratory toxicity based on Division of AIDS toxicity tables.

Design—This was a cross-sectional laboratory study of infants from birth to 6 months of age.

Methods—Blood samples were collected from a total of 561 infants and analyzed according to age categories similar to those in the 2004 Division of AIDS toxicity tables. Select chemistry and hematology parameters were determined and values compared with those in the toxicity tables.

Results—In the first 56 days of life, there were few graded toxicities except for neutropenia in 2 of 10 (20%) Ugandan and 13 of 45 (29%) Malawian infants at birth. After 7 days, about 20% of the infants in Uganda and Malawi would have been classified as having a neutropenia whereas 47% and 53% of those more than 2 months of age in Uganda and Malawi respectively, would have been reported as having an abnormal hemoglobin. Chemistry findings were not different from US norms.

Conclusions—These findings underscore the importance of establishing relevant local laboratory norms for infants.

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Keywords

Division of AIDS; normal laboratory reference values; non–HIV-exposed; toxicity tables; Ugandan; Malawian; infants

INTRODUCTION

There are physiologic variations in normal laboratory values among different race/ethnicity groups and by age.1,2 Because adverse event reporting is required in the conduct of clinical trials, a reliable and accurate tool is necessary to evaluate toxicities across populations. In many HIV clinical trials, the Division of AIDS (DAIDS), National Institute of Allergy and Infectious Disease/National Institutes of Health Toxicity Tables are being used to evaluate potential toxicities in multisite trials around the world, which include participants from diverse racial/ethnic backgrounds.

The laboratory values for the DAIDS Toxicity Tables reflect US-based laboratory references and were derived from multiple sources including pediatric reference text books, the National Cancer Institute tables, and reviews of relevant literature. The tables were reviewed by experts from several networks, some non-network experts and the Intramural Vaccine Research Center at National Institutes of Health (NIH). Medical Officers from the National Institute of Child Health and Human Development were also involved in determining the pediatric reference ranges.

Although use of standardized norms helps provide a common base for comparisons, reliance on laboratory norms based on data from US populations alone could lead to misclassification of normal values as “abnormal” among other populations such as in Africa and East Asia. This reliance could then result in substantive mislabeling of laboratory values; such as the reporting of high rates of “toxicity” with use of study drugs and making changes in management of study drugs based on an assumption of toxicity for laboratory values that may fall within the normal range in a given local setting. This is particularly important in phase I safety trials where there may not be “control groups” as comparisons.

Likewise, mislabeling potential candidates as ineligible for participation due to a laboratory “abnormality” could exclude numerous healthy volunteers at international sites from participating in clinical trials. Not only are there limited data comparing normal ranges for hematologic values among African children compared to Caucasian children, but there is also a paucity of data on relevant local reference chemistry norms in African infants.

The objectives of this study were to determine normal hematologic and selected blood chemistry values among healthy non–HIV-exposed infants during the first 6 months of life in Uganda and Malawi and to use this information to assess the site-specific proportion of healthy babies who would be labeled as having an “abnormal” or toxicity-graded laboratory value based on US reference norms using the 2004 DAIDS toxicity tables.3

METHODS

The study was carried out by investigators in Uganda and Malawi using 2 similar but separate protocols. Infants were recruited from Mulago Hospital, Kampala Uganda; and at Chilomoni, Limbe, and Ndirande government health clinics and Queen Elizabeth Central Hospital in Blantyre Malawi. The protocols were approved by the Johns Hopkins University Institutional Review Board and Ugandan and Malawian Institutional Review Boards.

Study Design and Recruitment Procedures

The 2 cross-sectional laboratory studies were conducted in Uganda and Malawi.

When an eligible mother–infant pair was identified, the mother was approached, and if she was willing to participate in the study, written informed consent was obtained from her before enrollment of her infant into the study. In both Uganda and Malawi, infants were recruited either at the time of birth before discharge of mothers from the hospital or at postnatal maternal–child health clinics. Infants were recruited to approximate the age groups that are reflected in the 2004 DAIDS Toxicity Table3 and were systematically enrolled with a goal of achieving a minimum of 30 participants in each group or a total of approximately 210–240 evaluable infants at each study site. In Uganda, infants were assigned to birth group (or day zero) if the sample was drawn within less than 24 hours after birth, whereas in Malawi, samples were assigned to an age group based on the calendar date the sample was drawn regardless of hours after birth. Though the definition for “Birth Sample” was different at the 2 sites during the study period, to achieve consistency, at the time of data analysis, Ugandan infants were reassigned an age group based on the calendar date the sample was drawn after birth rather than based on hours after birth.

Inclusion Criteria for Mother and Infant Uganda

Healthy term infants born after uncomplicated pregnancies to HIV-negative mothers (ie, mothers who tested HIV negative within 1 month before the day of enrollment and with documentation of HIV-negative status) were eligible. Full term non–HIV-exposed infants aged 0–6 months of age, with an axillary temperature <37.6°C, without life-threatening conditions, acute illnesses or major congenital anomalies, and with a birth weight of greater than or equal to 2.5 kg were enrolled to have a 1-time blood draw. Infants from multiple gestation births were excluded. Each infant was assigned to 1 of 8 - time interval/age groups namely; birth (day 0), 1–7 days, 8–21 days, 22–35 days, 36–56 days, 57–123 days, 124–159 days, and 160–200 days of life.

Malawi

Mothers, who had a negative HIV test done within 7 days of screening and with written documentation of HIV-negative status, were approached for recruitment of their infants. Full-term non–HIV-exposed infants, aged 0–6 months of age, without life threatening conditions, acute illnesses, or major congenital anomalies were enrolled to have a 1-time blood draw. Multiple gestation births were included, but infants were excluded if they had had a transfusion. There were no exclusions based on birth weight. Infants were assigned to 1 of 7 - time interval/age groups: birth (day 0), 1–7 days, 8–21 days, 22–35 days, 36–56 days, 57–123 days, and 124–190 days of life.

For mothers whose HIV status was unknown, pretest and post-test HIV counseling was offered along with HIV testing using standard rapid HIV assays. In Uganda, mothers had their HIV testing done in the antenatal clinic as part of the Prevention of Mother to Child Transmission program, during labor/delivery or at the time of enrollment of the infant. In Malawi, mothers were tested for HIV antibodies if they had expressed interest in the study during voluntary counseling and testing.

At the time of the scheduled blood draw of the infant, a source form containing basic demographic information such as age, sex, vital signs, a targeted history, and a physical examination (to rule out acute illnesses) was completed by a trained study staff. The study procedure required a 1 time visit and blood draw. However, in Uganda, if the infant was found to be sick when they were brought in for their scheduled appointments, they were seen for acute care and a new appointment date for the blood draw was given after the acute

illness had resolved. When an infant returned after the acute illness had resolved, they were assigned the correct age group based on their actual age at the time of the visit.

Laboratory Tests

Uganda—The Makerere University–Johns Hopkins University (MU-JHU) Core Laboratory was established in 1989, and provides clinical laboratory support for the research collaboration's clinical trials and observational studies at Mulago Hospital Complex. The MU-JHU Core Laboratory has been accredited by the College of American Pathologists since April 2003 and follows internationally recognized research ethics, Good Clinical Practices, and Good Clinical Laboratory Practice (GCLP) guidelines.

Phlebotomy was performed on infants via venipuncture, and a total of 2–3 mL of blood was collected: 1.0–1.5 mL in a 4.0 mL purple top (EDTA) Vacutainer tube, and 1.0–1.5 mL in a 4.0 mL red top serum Vacutainer tube (Becton Dickinson, Franklin Lakes, NJ), between 9 AM – 4 PM during working days. The blood was then transported from the Mulago Labor/ Delivery Ward (for the newborn babies) and from the postnatal clinic or research clinic (for babies up to 180 days old), to the MU-JHU Core Laboratory. The samples were received at the Core laboratory processing section, accessioned using unique laboratory accession codes, and tests were run on the same day. Results were made available to the mother within 2–3 hours.

For hematology, a complete blood count which included a 5-part White Blood Count (WBC) differential count and percentage (neutrophils, lymphocytes, monocytes, eosinophils and basophils) was performed. Infant serum was used to test for the following 4 chemistry analytes: total bilirubin, aspartate transferase (AST), alanine transferase (ALT), and creatinine. Other chemistry analytes tested that were analyzed depending on availability of extra sample included: albumin, alkaline phosphatase, amylase, urea (BUN), creatine kinase, chloride, bicarbonate (HCO_3), Gamma glutamyl transpeptidase (GGT), glucose, potassium (K), sodium, and uric acid.

The hematology tests were analyzed on the Coulter ACT 5 instrument (Beckman-Coulter, Inc, Fullerton, CA). The chemistry tests were analyzed on the COBAS Integra 400+ (Roche Diagnostics, Indianapolis, IN). Maternal rapid HIV assays were done using Determine, Statpak, and Unigold [Determine HIV ½ (Abbott Diagnostics, Abbott Park, IL); STAT-PAK (CHEMBIO Diagnostic Systems Inc, New York, NY); UniGold (Trinity Biotech PLC Company, Bray, Ireland)].

Malawi—In Blantyre, Malawi, the Johns Hopkins University–College of Medicine Research Project (JHU/COM RP) Laboratory provides laboratory support for research supported by the Johns Hopkins Bloomberg School of Public Health and the College of Medicine of Malawi. The JHU/COM Laboratory operates within the Queen Elizabeth Central Hospital in Blantyre, Malawi, and it follows internationally recognized research ethics codes including Good Clinical Practice and GCLP guidelines.

Infants who were eligible for participation had approximately 500 μL of whole blood collected into a 1.0 mL BD Microtainer EDTA (RAM) and 2.5 mL of whole blood collected into a 5.0 mL BD Vacutainer serum separation tube by venipuncture between the hours of 8:00 AM–4:00 PM. The blood was transported from each of 4 health centres in Blantyre and Queen Elizabeth Central Hospital to the JHU/COM RP Laboratory. The serum separation tube was used for the chemistry testing on serum, and the EDTA tube was aliquoted for a complete blood cell count. The complete blood count was reported using the same type of instrument as that used in Uganda. Chemistry tests were analyzed on a Beckman Coulter CX5 Chemistry analyzer (Beckman-Coulter, Inc).

For maternal rapid HIV assays, Determine HIV 1/2 (Abbott Diagnostics) and OraQuick HIV 1/2 (Orasure, Bangkok, Thailand) kits were used.

At both Uganda and Malawi sites, total bilirubin, ALT, AST, and creatinine were run but at the Uganda site, additional reference range analysis for CK, GGT, glucose, BUN, and other chemistries were run if there was sufficient sample. A comparison with the DAIDS tables was not done for several chemistry parameters including creatinine, alkaline phosphatase, ALT, AST, bicarbonate, albumin, bilirubin >14 days, creatine kinase, lipase, and phosphate because the DAIDS toxicity tables use “upper limit of normal” instead of absolute values for these particular laboratory parameters.3

Statistical Methods

The NIH DAIDS Tables for Grading the Severity of Adult and Pediatric Adverse Events Publish Date: December, 2004 Version 1.0, and their defined age categories were used to report the proportions of healthy Ugandan and Malawian non–HIV-exposed infants aged 0–6 months with hematologic and chemistry values that would be classified as normal or abnormal. The abnormal values were graded as grade I (mild), grade II (moderate), grade III (severe), or grade IV (life threatening) levels of toxicity.

The emphasis of the current article is to assess the validity and utility of the DAIDS Toxicity pediatric reference limits for assessing toxicity among Ugandan and Malawian infant populations. To assess the adequacy of a given reference interval, Clinical Laboratory Standards Institute4 guidelines require sample collection from at least 20 reference individuals and performance of a formal outlier test. If more than the critical number of individuals for a specified sample size evaluated is outside the reference interval, then the Clinical Laboratory Standards Institute guidelines recommend that the user should re-examine the analytical procedures used, consider possible differences in the biological characteristics of the 2 populations sampled, and consider developing their own reference interval. The critical numbers of individuals beyond which the DAIDS Toxicity reference limits reference interval should be considered for rejection have been obtained for the age-specific sample sizes employed in this study using methodology similar to that of a Lot Quality Assurance Sampling methodology.5

We have computed and presented these critical values for the subgroup sample sizes for each of the laboratory parameters. These critical values indicate that having this number or more observed individuals in the subgroup should prompt us to consider possible differences in the biological characteristics of our sample population and that of the DAIDS population, and thus consider whether we should be using site-specific reference intervals for this sampled population and/or use upper and lower limits of normal based on local site data to calculate toxicity grading. All statistical analyses were done using R and Stata statistical packages.6,7

RESULTS

The study was carried out from August 2006 to May 2007. A total of 254 healthy full-term infants born to HIV-negative mothers at Mulago Hospital in Uganda and 307 infants in Blantyre Malawi were enrolled in the study. Data from both sites were analyzed separately and are presented in this report.

Age groups, birth weights, and sex for infant enrollees at each site are shown in Table 1. There were almost equal numbers of male and female infants enrolled into the study. Mean birth weight was 3.2 kg and 3.1 kg in Uganda and Malawi, respectively.

As shown in Tables 2 and 3, there were few abnormal grade values noted for hematology assays in the first 56 days of life when using the 2004 DAIDS Toxicity Tables, except for neutropenia, where in Uganda 2 of 10 (20%) and in Malawi 13 of 45 (29%) would present with any degree of neutropenia on the first day of life. After 7 days, about 20% of healthy infants at both sites, 40 of 254 (15.7%) and 56 of 288 (19.4%) in Uganda and Malawi, respectively, would have been classified as having a low neutrophil count, but with less than 5% showing a moderate to severe grade of neutropenia. After 2 months, about half, 42 of 90 (47%) and 39 of 73 (53%) of healthy infants at the sites in both Uganda and Malawi, respectively, would have been reported as having an abnormal hemoglobin using the 2004 DAIDS Toxicity Tables.

Among the infants, 8 or more weeks (≥57 days) of age, severe (grade 3 or 4) toxicities were less common but also evident, with about 2 of 90 (2%) of Ugandan and 6 of 73 (8%) of Malawian healthy infants showing a grade 3 decreased hemoglobin; and 1% with a grade 4 decreased hemoglobin at both sites. After 7 days of age, 7 of 179 (4%) and 5 of 206 (2.5%) of healthy infants at the Uganda and Malawi sites, respectively, would be labeled as having a grade 3 or 4 neutropenia using the 2004 DAIDS Toxicity Tables as normal reference ranges.

In contrast, chemistry findings were generally not different between Ugandan, Malawian, and US infants when using the 2004 DAIDS Toxicity Tables. Specifically no differences were found for renal and liver function tests and most electrolytes. Approximately one third of Ugandan infants showed a grade I low sodium, but this may have been related to the reference ranges of the laboratory chemistry machine. In addition, 32% of healthy Ugandan infants less than a month of age would have been classified as having an abnormal low blood glucose based on the DAIDS Toxicity Tables.

DISCUSSION

In this study of laboratory values in healthy non–HIV-exposed Ugandan and Malawian infants in the first 6 months of life, we found that about 1 in 5 infants would have been classified as having a neutrophil toxicity and about half of healthy infants would have been classified as having a hemoglobin toxicity using US-based DAIDS Toxicity Tables. These findings suggest caution in terms of interpretation of toxicities versus anticipating some expected laboratory value variations at international sites compared with US sites.

Prior studies comparing normal ranges for hematologic values among African children and US or European children are limited, but the data available support that there are variations in the normal ranges of laboratory values across different race/ethnic groups.8–10 A report from Uganda looking at hematological findings from a cross-sectional household survey of infants through adults in a rural Eastern parish found a median hemoglobin of 10 g/dL in children <1 year of age and 10.8 g/dL among children aged 1–5 years.11 This mean hemoglobin level is substantially lower than mean values seen in US infants and young children.

Likewise, a study by Neser12 carried out in the 1960s in Pretoria, South Africa, compared white blood cell counts for white, black, and Indian children ages 6–15 years. This study found that even in the same environment, for children 7–11 years of age, the mean total white blood cell count was statistically significantly different, with Indian children having the highest total white count followed by white, with the lowest mean value among black children. Among those aged 12–15 years, similar patterns were noted of lower white blood cell counts among black children and higher values among Indian children. Likewise, in this South African study, neutrophil counts followed similar patterns by race/ethnicity; and

declines in absolute neutrophil counts (ANC) by age were most striking among black children. Furthermore, there are a number of reports in the literature of what has been termed “benign ethnic neutropenia and leucopenia” among 25%–50% of healthy persons of African descent and among some ethnic groups in the Middle East.13

Use of normal reference values is critical in the conduct of clinical trials. More recently, hematologic studies comparing normal values of HIV-exposed noninfected infants have also demonstrated variations in hemoglobin and neutrophil count between African and European infants born to HIV-infected women based on studies from Ethiopia, Malawi, and the European Collaborative Study.14–16 Given exposure to peripartum antiretrovirals, it is possible that the noted differences could be attributed to drug exposure. However, in these uninfected infants, the laboratory toxicities such as neutropenia were observed in clinically healthy infants and the neutropenia resolved on its own without change in drug regimen, thus suggesting a background lower neutrophil count in this population that is not a drug-related toxicity. In the Ethiopian study, it was observed that among infants born to HIV-positive breastfeeding women, in which infants were randomized to receive either only birth dose or birth dose and nevirapine (NVP) through 6 weeks, infants born to HIV-positive nonbreastfeeding women, and infants born to HIV-negative women, the mean ANC for the 3 cohorts at all ages was lower than the respective mean ANC reported in the United States.

Our results support earlier findings from studies in South Africa12 and more recent studies in Ethiopia and Zimbabwe.14,17 In Zimbabwe, in a study to establish normal hematologic values in black Zimbabwean infants and to quantify the apparent prevalence of relative neutropenia in the population, HIV-uninfected healthy infants born to HIV-uninfected women were evaluated at birth, 10 days, 6 weeks, 3 months, and 4 months of life. The study demonstrated that the mean ANC values for Zimbabwean infants were less than 50% of the accepted normal values for US infants.17

A study in Malawi assessing HIV-exposed but uninfected infants who received either single-dose NVP alone or combined with 1 week of zidovudine reported that the mean hemoglobin varied between 9.8 and 10.7 g/dL for infants aged 6 weeks and 18 months. US norms reported for the same age range among a primarily white US population were generally 1 gm/dL higher at each age grouping except birth.18

Lower neutrophil counts have also been noted in 3 recent perinatal studies conducted in resource-limited African settings in Ethiopia and Malawi, when compared with infant neutrophil count norms based on US populations.14,19 A report from a perinatal trial in Lilongwe, Malawi, found that when using the DAIDS Toxicity Tables, 86 of 206 or 41.7 % of HIV-exposed uninfected infants enrolled in a perinatal prevention trialwould be labeled as having a neutrophil toxicity with 25 of 206 or 12.1% having a grade 3 or 4 toxicity at birth.19

Likewise, in a perinatal trial of infant NVP prophylaxis in Addis Ababa, Ethiopia, statistically significantly high rates of infant neutropenia were noted at ages 6 and 14 weeks when compared with US norms for the same age groups. The mean ANC values for Ethiopian infants were about one-third lower than infant ANC norms using the DAIDS Toxicity Tables.14

Bunders et al20 investigated racial immunohematological differences in HIV-uninfected children with or without HIV exposure in Uganda and Europe. They compared cross-sectional data from 1633 children in Uganda to data on 1959 children residing in Europe [black (n = 604) and white children (n = 1355)], and noted that during infancy, the total lymphocyte count, $CD4^+$, and $CD8^+$ counts were lower in Ugandan children than black

European children. Of interest, in Ugandan children, $CD4^+$ counts and neutrophil counts were also lower than in European children born to Ugandan mothers.

In contrast to hematologic findings, our study did not show much variation in the biochemical values for infants in Malawi and Uganda compared with pediatric values in the US DAIDS toxicity tables, although other investigators have demonstrated some differences in infant blood chemistries by race ethnicity.21

We believe that these lower hemoglobin found among healthy Ugandan and Malawian infants do not reflect evidence of physiologic dysfunction because the babies were healthy, born after uncomplicated pregnancies, which should ensure adequate in utero stores of iron. In addition, they were being breast fed which would allow for on-going supply of bio-available iron. Likewise, the lower neutrophil ranges seen in this study have been well-documented in earlier studies of black African populations compared with white populations and have not been associated with any harmful effects.

Because adverse event tracking and reporting is an integral part of clinical trials, there is need for accurate identification of real versus apparent adverse events (AEs). In a study to determine costs for AE procedures for a large HIV perinatal trial in Uganda, Chou et al22 demonstrated that costs associated with AE investigation and reporting procedures represented 32% of all study expenses irrespective of the grading of the AE. This calls for better planning for AE reporting systems and the need to avoid reporting apparent AEs if they are actually a normal variant in the population.

These findings demonstrate potential shortcomings in the use of the US-based reference ranges when assessing laboratory toxicities in international settings. However, in the absence of local laboratory references for hematological and chemistry values in randomized clinical trial, where comparison between study arms is the desired end point, the use of DAIDS toxicity scales in international sites can provide useful data. The tables allow for comparisons of event rates by study arms; and standardized comparison of event rates across sites.

Relative Limitations and Strengths of These Normal Values Findings

There are certain caveats to these laboratory findings. First, this was a cross-sectional study done at only 2 international urban sites in Uganda and Malawi, and thus data may not be generalizable nationwide or to other international settings. Second, by age group, the samples sizes are relatively modest. Given these limitations, there are also a number of strengths of the study which include the fact that the data are from a sample of healthy non–HIV-exposed infants in both Uganda and Malawi and provide local site data that can be used in clinical trials to evaluate adverse events within the local context. Likewise, the laboratories used in this study are both regularly monitored and take part in ongoing proficiency testing and GCLP training as part of DAIDS/NIH Quality Assurance laboratory programs. This quality assurance helps ensure the validity and reliability of the laboratory values generated in this infant laboratory study.

These findings from both Malawi and Uganda suggest that caution needs to be used when interpreting laboratory “adverse events” for African infants based on US norms, particularly for hemoglobin and ANCs. Specifically, the differences found could potentially result in mislabeling normal hematologic or chemistry values as abnormal based on reference ranges from a different race/ethnicity cohort such as in the United States. This misinterpretation could inadvertently lead to exclusion of healthy infants from studies, based solely on “abnormal” laboratory values. Likewise it could result in the overestimation of toxicities judged as possibly attributed to study drugs or concomitant medications within a trial.

Based on the hematologic and chemistry ranges for healthy Uganda and Malawi infants found in this study, we recommend that each trial site carry out normative laboratory studies or use already available data on infants in their local settings to assess whether the laboratory values obtained in the trial are within the normal range. A further consideration in modifying the DAIDS Toxicity Tables would be to convert all grading to upper and lower limits of normal based on local norms as is already being done for most of the chemistry values.

In conclusion, these findings underscore the importance in the conduct of international pediatric clinical trials to gather relevant local norms for infant hematologic values to avoid mislabeling laboratory values of healthy infants taking part in a study as abnormal or assuming possible drug toxicity based on inaccurately describing a normal value as representing a toxicity grade.

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TABLE 1

Age Groups and Demographic Characteristics of Infants

Age Category (days)	Uganda, n = 254 n(%)	Malawi, n = 307 n (%)
Birth day 0	10 (3.9)	43 (14.0)
1–7	66 (25.9)	41 (13.4)
8–21	26 (10.2)	41 (13.4)
22–35	30 (11.8)	40 (13.0)
36–56	32 (12.6)	62 (20.2)
57–123	33 (12.9)	38 (12.4)
124–159	25 (9.8)	124–190 days: 42 (13.6)
160–200	32 (12.6)	—
Birth weight (mean ± SD) (Kg)	3.2 ± 0.44 IQR (2.9–3.5)	3.1 ± 0.48 IQR (2.8–3.4)
Sex		
Female	129 (50.8)	156 (51.0.)
Male	125 (49.2)	150 (49.0)
Total	254	306*

*One infant from Malawi did not have sex recorded, but data were analyzed within the appropriate age group based on age.

TABLE 2

Numbers and Percentage of Healthy Infants With Normal Versus Abnormal Laboratory Values by Severity Level Using DAIDS Toxicity Tables 2004 and Age Ranges—Kampala Uganda

		DAIDS Adverse Event Severity Grading						
CBC	**Infant Age Groups**	**Normal**	**Mild**	**Moderate**	**Severe**	**Life Threatening**	**Total**	**d'***
Hemoglobin, g/dL	Birth 21 days	96 (95%)	3 (3%)	2 (2%)	0	0	101	9
	22–35 days	29 (97%)	0	1 (3%)	0	0	30	4
	36–56 days	31 (3.90%)	2 (6.10%)	0	0	0	33	4
	≥57 days	48 (53%)	25 (28%)	14 (16%)	2 (2%)	1 (1%)	90	8†
Absolute neutrophil count, × 10^3/μL	Birth	8 (80%)	1 (10%)	0	1 (10%)	0	10	2†
	1–7 days	62 (95%)	2 (3%)	0	1 (2%)	0	65	6
	>Than 7 days	144 (80.40%)	16 (8.90%)	12 (6.70%)	6 (3.40%)	1 (0.60%)	179	14†
Platelets decreased × 10^3/μL	NA	248 (97.60%)	2 (0.80%)	2 (0.80%)	1 (0.40%)	1 (0.40%)	254	19
White blood cell decreased × 10^3/μL	NA	254 (100%)	0	0	0	0	254	19
Chemistry								
Total bilirubin, mg/dL	≤14 days nonhemolytic	84 (100%)	0	0	0	0	84	8
	<14 days hemolytic	84 (100%)	0	0	0	0	84	8
Sodium high, mEq/L	Not applicable	204 (99.50%)	1 (0.50%)	0	0	0	205	16
Sodium low, mEq/L	Not applicable	135 (65.50%)	70 (34.50%)	0	0	0	205	16†

For each group The DAIDS Adverse Event grading categories were based on the values in the DAIDS Toxicity Tables December, 2004 Version 1.0.

Nineteen (19) infants from Malawi did not have ANC results. There were 14 clotted samples and for 5 samples the machine could not read neutrophils.

*Critical values indicate that having this number or more observed individuals in the subgroup should prompt us to consider possible differences in the biological characteristics of our sample population and that of the DAIDS population, and thus, consider whether new reference intervals should be developed for this sampled population.

†Indicates that the observed number of individuals outside of the reference intervals for a given age group is equal or greater than the critical number that is consistent with the expected DAIDS tables' population distributional properties.

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TABLE 3

Numbers and Percentage of Healthy Infants With Normal Versus Abnormal Laboratory Values by Severity Level Using DAIDS Toxicity Tables 2004 and Age Ranges—Blantyre Malawi

		DAIDS Adverse Event Severity Grading						
CBC	**Infant Age Groups**	**Normal**	**Mild**	**Moderate**	**Severe**	**Life-Threatening**	**Total**	**d'***
Hemoglobin, g/dL	Birth 21 days	107 (87.7%)†	5 (4.10%)	7 (5.70%)	2 (1.60%)	1 (0.80%)	122	10†
	22–35 days	33 (84.60%)	3 (7.60%)	2 (5.10%)	1 (2.50%)	0	39	4†
	36–56 days	55 (93%)	3 (5%)	1 (2%)	0	0	59	6
	≥57 days	34 (46.60%)	20 (27.4%)	12 (16.4%)	6 (8.20%)	1 (1.40%)	73	7*
Absolute neutrophil count, × 10^3/μL	Birth	32 (71.10%)	5 (11.1%)	2 (4.40%)	3 (6.70%)	3 (6.70%)	45	5†
	1–7 days	36 (97%)	1 (3%)	0	0	0	37	4
	>Than 7 days	164 (79.60%)	26 (12.6%)	11 (5.30%)	4 (2%)	1 (0.50%)	206	16*
Platelets Decreased, × 10^3/μL	NA	262 (89.4%)	9 (3.10%)	18 (6.1)	4 (1.40%)	0	293	21†
White blood cell decreased, × 10^3/μL	NA	293 (100%)	0	0	0	0	293	21
Chemistry								
Total bilirubin, mg/dL	≤14 days nonhemolytic	73 (98.60%)	0	0	0	1 (1.40%)	74	7
	<14 days hemolytic	70 (98.60%)	0	0	0	1 (1.40%)	71	7
Sodium high, mEq/L	Not applicable	271 (97.50%)	5 (1.80%)	0	0	2 (0.70%)	279	20
Sodium low, mEq/L	Not applicable	261 (93.50%)	16 (5.70%)	2 (0.70%)	0	0	279	20

For each group The DAIDS Adverse Event grading categories were based on the values in the DAIDS Toxicity Tables December, 2004 Version 1.0

Nineteen (19) infants from Malawi did not have ANC results. There were 14 clotted samples and for 5 samples the machine could not read neutrophils.

*Critical values indicate that having this number or more observed individuals in the subgroup should prompt us to consider possible differences in the biological characteristics of our sample population and that of the DAIDS population, and thus, consider whether new reference intervals should be developed for this sampled population.

†Indicates that the observed number of individuals outside of the reference intervals for a given age group is equal or greater than the critical number that is consistent with the expected DAIDS tables' population distributional properties.