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Pharmacokinetic Studies in Infants Using Minimal-risk Study Designs

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Abstract

Infants are therapeutic orphans. Many drugs used in infants are used “off-label”, increasing the risk of drug toxicity and suboptimal efficacy in this vulnerable population. This knowledge gap in clinical pharmacology is partly attributed to challenges associated with conducting clinical trials in infants. Consequently, there is a need for novel and efficient study designs more suited to this population. Available literature describing the use of minimal-risk approaches to characterize the pharmacokinetics (PK) of drugs in infants was critically reviewed. Population PK approach with sparse sampling was found to be well established. Other, more recent alternatives, like dried blood spots sampling, opportunistic design, and the use of non-blood matrices are promising strategies with an increasing number of applications in infants. Physiologically based pharmacokinetic modeling provides valuable insight in drug disposition but still needs more prospective validation. Increasing experience with these methods provides understanding of their strengths and limitations and will help improve the design of future PK studies in infants.

Keywords

Clinical trial; dried blood spots; infants; pharmacokinetics; population pharmacokinetics; scavenged sampling; sparse sampling; ultra; low-volume drug assays

Over the past 20 years, investigators and regulatory agencies have prioritized research to increase the quality of evidence in pediatric clinical pharmacology. During this time period, legislative initiatives in the United States have resulted in approximately 436 pediatric studies that have contributed to labeling changes (1). In contrast to the success seen with children, infants continue to be excluded from clinical trials because of the challenges inherent to performing clinical trials in this vulnerable and often critically ill population. Those challenges include: low study consent rates; limited blood volume available to conduct pharmacokinetic (PK) studies; difficulty in obtaining blood from infants; limited use of sensitive, low-volume drug concentration assays; and a lack of expertise in pediatric modeling and simulation. Consequently, there is a need for novel and efficient study designs

more suited to the infant population. Minimal-risk study designs, including ultra-low-volume assays, PK modeling and simulation, opportunistic drug protocols, and the use of alternative biological fluids (other than plasma) to evaluate drug disposition, can reduce the volume and frequency of blood sampling. These study designs also allow decreasing the number of subjects required to describe the PK and dosing requirements of drugs. This review will describe the use of such minimal-risk approaches to characterize the PK of drugs in infants.

1. Microanalytical methods

Ultra-low-volume drug assays

The blood volume of preterm and term infants is ~ 80 mL/kg; thus, a premature infant weighing 500 g has a circulating blood volume of roughly 40 mL (2, 3). Traditional PK sampling volumes (1–2 mL per sample) required to measure drug concentrations are impractical in this population. The volume of blood required for sampling can be minimized, however, by using ultra-low-volume assays. These assays are capable of quantifying drugs in plasma samples of <50 μ L, a volume that requires collection of only 100 μ L of whole blood per sample. Advances in bioanalytical technology have allowed such reductions in sample volume while maintaining acceptable levels of detection, accuracy, and precision. Bioassays in plasma volumes as low as 20 μ L have been validated for drugs commonly prescribed in infants, including acetaminophen, phenytoin, and ampicillin (4-7). Multiplex assays leverage this technology further through simultaneous detection of multiple compounds, as well as parent and metabolite products, from a single plasma sample (4-7), thereby maximizing the data extracted from each PK sample.

Ultra-low-volume assays have been used to gain drug disposition information in infant PK studies (Table 1). As an example, the PK of meropenem was characterized in 188 preterm and term infants using drug concentrations measured in 50 μ L of plasma (8). A multiplex plasma assay (50 μ L) including meropenem, piperacillin-tazobactam, acyclovir, metronidazole, and ampicillin was used to measure drug concentrations for two clinical trials in premature infants receiving metronidazole, piperacillin-tazobactam, and acyclovir (6, 9, 10). The information obtained from these samples was maximized by capturing multiple drugs, which were then included in the PK analysis.

Dried matrix spots

Dried matrix spot (DMS) samples are samples of biological fluids collected on blotting paper. Each sample requires minimal volumes ranging from 5–30 μ L and can include whole blood, or other body fluids (dried blood spots [DBS]), urine (dried urine spots [DUS]), plasma (dried plasma spots [DPS]). DBS are well established for screening of inborn errors of metabolism in newborns (11) and are increasingly used clinically for therapeutic drug monitoring (12, 13). The use of DMS for drug development, however, has only been embraced by industry and government within the past 5–7 years (14). Its uptake may be partially attributed to the availability of sensitive instruments that can reliably measure drug concentrations in minimal amounts of sample (14-16). This technology is particularly attractive for clinical pharmacology studies in infants. Dried matrix spots are also appealing

due to minimal sample preparation, potential room-temperature storage, reduced costs, and convenient shipping (13, 17, 18).

DBS samples were used successfully to characterize the PK of metronidazole in premature infants in two studies (19, 20) (Table 1). The use of DBS as the biological matrix for PK analysis did not introduce bias in PK parameter estimates in either case. In one of these studies, paired samples of plasma and DBS were collected at each sampling time point; DBS metronidazole concentrations were within 85% of plasma concentrations across the plasma concentration range (19). This finding suggests *in vivo* metronidazole partitioning into red blood cells, which confirms prior *in vitro* observations (21). The extent of drug partitioning into red blood cells will influence differences observed between plasma and whole blood concentrations. Because plasma drug concentrations are often linked to efficacy or pharmacodynamic endpoints, comparability between plasma and DBS (whole blood) drug concentrations is critically important. On occasion, DBS drug concentration measurements can be corrected if reliable hematocrit information is collected from participants in clinical trials. This possibility was explored in a PK study of caffeine in newborns (17). After determining the effect of hematocrit on drug concentration *in vitro*, hematocrit-adjusted concentrations were incorporated into the PK model. In this study, caffeine PK parameters were similar to previously reported plasma-derived estimates.

Alternatively, plasma has also been sampled on filtered paper as DPS. A validated, quantitative bioassay for paroxetine was recently reported requiring 20 μ L of plasma (22). This DPS assay was then applied in a study involving adult healthy volunteers and showed good correlation between DPS and traditional plasma samples (22). To our knowledge, DPS has not been used in PK studies involving infants. Similar to DBS, DPS represents an interesting alternative to traditional plasma sampling because of its low volume as well as convenient storage and shipping options.

It is clear that DMS PK sampling offers advantages relative to traditional sampling in early-phase trials involving premature infants. However, certain limitations should be considered. First, as discussed above, DBS analysis requires additional analysis to take into account red blood cell drug distribution. A second concern involves drug stability: storage at room temperature is often cited as one of the biggest advantages of DBS; however, some highly unstable drugs might undergo degradation during the drying process, which may take 2 hours at room temperature (23, 24). Moreover, long-term stability should be evaluated to take into account potential extraction issues or degradation after long-term storage. Another important consideration is standardization of the collection procedure, especially for the spotting technique of whole blood on filter paper. Homogeneity of the blood spot is critical for providing reliable results. The importance of adequate training for collection procedures was illustrated in the caffeine trial cited above in which 12% of DBS samples were deemed unacceptable due to coagulated blood spots, merged samples, or insufficient samples. However, the number of rejected samples in this trial appears higher than the usual rejection rates reported in the Newborn Screening Program using a similar DBS sampling technique (0.1–1%) (25). Lastly, because DMS is a relatively novel tool in clinical pharmacology, the greatest limitation is the lack of standardization and Food and Drug Administration (FDA) guidance on this technique. Critical steps such as spotting of biological fluid on the filter

paper, sample extraction (punching of the card), hematocrit considerations, and the bioanalytical assay itself can affect PK estimates and therefore require validation.

2. Pharmacokinetic modeling and simulation

Modeling and simulation can be used to predict or characterize dose-concentration and dose-response relationships of a drug. This approach can be very informative in the design of clinical trials by identifying optimal PK sampling time points and dosing regimens. While allometric scaling is widely used for dose adjustment in children over 6 years of age (e.g., child dose = adult dose*[child weight /70kg]^{0.75}) (26, 27), PK modeling and simulations provide more reliable predictions in infants by integrating semi-physiologic functions characterizing drug disposition (28).

Population PK modeling and sparse sampling

Population PK modeling evaluates PK parameters at a population level based on observed PK data in a group of individuals. This approach identifies sources of inter- and intra-individual variability in drug disposition and allows for identification of covariates that can explain inter-individual variability. An advantage of population PK modeling is its ability to rely on sparse sampling, a strategy in which a small number of samples per child (often only 2–3) are collected from a large number of individuals. A sparse sampling approach allows for fewer blood draws per child and flexibility in sampling times. Population modeling is a well-established methodology in infants, and the number of PK trials using this approach is increasing (29, 30). As demonstrated in Table 1, population PK analyses and sparse sampling (defined as 3 samples per subject) in infants are well represented in the literature.

Physiologically based PK modeling

Physiologically based PK modeling (PBPK) modeling relies on a mathematical model characterizing drug disposition within a system of interconnected virtual organs. PBPK models are mathematical tools that integrate drug-specific (e.g., metabolism, protein binding) and system-specific (e.g., organ size, blood flow) information to predict the effect of different factors (e.g., age, genetic variants, disease) on drug exposure (31, 32). This approach offers advantages over traditional PK models by: 1) incorporating physiologic developmental changes into the model to account for age effects on dosing; 2) predicting drug concentrations in the site of action; and 3) providing a mechanistic understanding of drug disposition (33). As a result, the models are able to better predict systemic drug exposure in both plasma and tissue and, by inference, discern the appropriate drug dose. Optimization of therapy in this fashion has the potential to improve both efficacy and safety. PBPK models can also be used to identify optimal PK sampling time points. For example, in preparation of a clinical trial, plasma concentration–time profiles were simulated in children (2–18y) using a PBPK model for the parent drug and its active metabolite. Sampling time points were optimized based both on the minimization of relative standard errors of PK parameters and clinical constraints (34). This step is critical to ensure success of pediatric clinical trials due to the limitations associated with the number and volume of PK samples that can be collected from children. This is especially important in children <2 years of age, who have limited blood volume for PK sampling, but who undergo the greatest changes in

developmental processes affecting drug disposition. Limitations include sparse systems-specific data in children of the enzymes and transporters responsible for drug metabolism and transport, respectively. While some data describing the ontogeny of drug metabolizing enzymes in infants have been published (35, 36), there is still incomplete information about some cytochrome P450 profiles across the age range (33). For example, in a PBPK model describing theophylline and midazolam PK, a linear clearance (i.e. first order elimination) was assumed in all age groups because of lack of enzyme activity data on CYP1A2, CYP2E1 and CYP3A4 (37). PBPK models that incorporate these data may lack precision as demonstrated by the under prediction of oral clearance of midazolam in infants resulting from missing information on intestinal metabolism by CYP3A (28). Lastly, the complexity of evaluating the inputs and developing the PBPK model requires specialized skills that are rare in pediatrics.

Predictions from PBPK models have been used successfully to characterize the PK of several drugs in infants, including midazolam, caffeine, paracetamol, morphine, fentanyl, theophylline, and levofloxacin (28, 31, 37). These examples used available drug concentration data to evaluate adequacy of the models and showed good agreements between PK predictions and previously published data. However, these predictions were not confirmed with prospective clinical trials (Table 1). This step is critical because the accuracy and precision of PBPK models are dependent on the drug evaluated, route of administration, population of interest, and assumptions of inter-individual variability (38-40). Evaluations of four pediatric submissions to the FDA made between 2009 and 2011 showed that PBPK models under-predicted drug clearance in children (33), and the FDA Advisory Committee for Pharmaceutical Science and Clinical Pharmacology in 2012 underscored the importance of human data to validate PBPK models (41).

Regulatory agencies strongly encourage the use of modeling and simulation in pediatric drug development (42, 43). Population PK is the preferred approach in situations where few patients and sparse data are available (44). There is a lack of regulatory guidance for PBPK modeling, however, and it is rarely included in support of submissions of drug applications to regulatory agencies (32).

3. Opportunistic PK studies

Although many drugs in children are understudied, they are still widely used. Opportunistic studies are designed to collect PK samples for a drug of interest that is already prescribed per standard of care. In many cases, samples can be collected at times of routine lab draws, eliminating the need for additional phlebotomy. This approach offers several advantages over traditional PK studies; most notably improved consent rates because parents may be more willing to enroll their children given that their child is not exposed to additional drug therapy and, that the investigational drug is, presumably, providing some therapeutic benefit. Moreover, opportunistic design allows collection of PK information in children who are representative of the target population to be treated with the drug. Challenges of opportunistic studies relate to the sparse sampling strategy that they often employ. Moreover, opportunistic PK sampling allows less control over sampling time, PK samples might therefore not represent the whole dosing interval. As a consequence, sparse sampling

requires larger numbers of participants (e.g., multi-center trials) and specialized expertise in modeling.

Two recent publications reported oseltamivir PK in term and preterm infants using an opportunistic design in the context of nosocomial exposure to influenza A (H1N1) (45, 46). Oseltamivir was administered for treatment or prophylaxis, and a protocol was rapidly implemented for the collection of PK samples in the context of standard-of-care therapy. Investigators collected a single PK sample in 20 infants. Population PK analysis suggested that premature infants required half the dose needed to treat older children because of underdeveloped renal function and hepatic metabolizing enzymes (45). Another PK model for oseltamivir was developed using four samples per infant (46). These data confirmed the results from the original population PK model for preterm infants 37 weeks postmenstrual age (PMA) but found that a lower dose than originally recommended in the previous trial was adequate for infants > 37 weeks PMA (46).

Another example of an opportunistic design is the ongoing Pediatric Opportunistic PK Study (POPS) (clinicaltrials.gov NCT01431326). This multi-center trial is evaluating the PK of over 20 off-patent, understudied drugs that are administered to children per standard of care. PK samples (e.g.: plasma, urine, cerebrospinal fluid, DBS) are collected at the time of routine body fluid collection. Currently, more than 600 children from 24 sites have been enrolled. Enrollment for ampicillin, one of the drugs of interest in POPS, has been completed with 73 infants. The population PK analysis demonstrated the importance of PMA in ampicillin clearance and resulted in dosing recommendations (47). The PMA-based dosing was superior to ampicillin doses currently recommended in popular pediatric handbooks (Neofax, Harriet Lane) in regards to the target achievement rate.

The use of scavenged samples collected from surplus blood or plasma from laboratory tests performed for routine medical care represents another opportunistic approach. These left-over samples can be used to measure drug concentrations within the context of a research protocol. Scavenged PK sampling is an attractive method to use in infant clinical trials because it increases the feasibility of biological sample collection by reducing the volume of blood collected and the number of vascular punctures. This approach can be combined with traditional PK samples to characterize the drug concentration-time curve.

Scavenged samples have been used successfully to characterize the PK of antimicrobials in premature infants. Population fluconazole PK was described based on 357 PK samples (140 [39%] scavenged) from 55 infants (48). Analysis showed that scavenged samples introduced < 4% bias to the parameter estimates. Results of this study led to dosing recommendations for a phase III, randomized, placebo-controlled trial of fluconazole prophylaxis in premature infants (clinicaltrials.gov NCT00734539). The PK of metronidazole in premature infants was also characterized successfully using scavenged samples (10). Based on 116 PK samples, of which 90% were scavenged, a PK model was developed (10). Concentrations from scavenged samples were more variable and were associated with a 30% underestimation of concentration compared to timed samples (10). However, once this bias was incorporated into the model, PK estimates were comparable to estimates from prior studies (49, 50). The results from this study led to new dosing recommendations based on

PMA (10). In another example, a PK study of piperacillin using scavenged samples allowed for the identification of covariates for PK model development in premature infants. However, the PK model demonstrated a large residual variability (73.8% coefficient of variation) and was not used for dosing recommendations because of unreliable parameter estimates (9).

The higher variability in concentrations from scavenged samples in the metronidazole and piperacillin examples above highlights two main limitations in scavenged sampling: 1) unstandardized storage conditions and 2) inaccurate recording of sampling time, which can affect drug concentration measurements. The degree to which drug concentration measurements are affected depends on the stability and half-life of the drug and requires an initial study to correlate scavenged sample concentrations with prospectively collected PK sample concentrations.

Opportunistic studies have proven successful for some drugs commonly used in infants, although scavenged sampling studies suggest the importance of considering drug physicochemical properties (e.g., stability) to ensure success. These experiences also highlight that collection of concurrent traditional PK samples and information on storage conditions will strengthen the scavenged sampling approach.

4. Alternative biological matrices

Biological matrices other than plasma are an attractive source of PK data in infants because they avoid phlebotomy in this vulnerable population. These matrices include readily accessible body fluids such as saliva or exhaled breath (51-55).

Saliva

Drug distribution in saliva depends on several factors, including the drug's physicochemical properties and saliva characteristics (56-59). According to basic principles of drug distribution, saliva concentration is expected to be higher for lipophilic, low-protein-bound, and non-ionized drugs. Physiologic factors such as saliva flow rate and pH may also affect saliva drug concentration. A rapid saliva flow rate has been shown to decrease variability in saliva/plasma ratio compared with a slow saliva flow rate (60). Consequently, stimulated saliva might be preferred over resting saliva (61). Saliva is typically collected using a cotton swab or capillary pipette (51, 61, 62). This less-invasive sampling technique can improve consent rates for PK studies (63). However, because saliva drug concentrations are a function of multiple factors and the correlation between saliva and plasma concentrations are described for only a few drugs, the use of saliva to describe PK remains limited.

PK studies of metronidazole and caffeine using saliva concentration have been reported in infants. The metronidazole study showed high correlation between saliva and plasma concentrations ($r=0.82$) in a cohort of 20 children but included only one infant (64). In preterm infants, caffeine saliva and plasma concentrations were similar at a single time point in both matrices ($r=0.89$) after stimulation of saliva production with citric acid (61). Lastly, saliva amoxicillin and ampicillin concentrations were also reported in infants, but no saliva/plasma ratio was described, limiting the applicability for saliva-based clinical trials (62).

There are more data describing saliva PK in children and adults. In these older populations, PK estimates were consistent in both matrices for busulfan and artemisin (51, 53). These two studies showed good correlation between plasma and saliva concentrations (busulfan $r=0.96$ and artemisin $r=0.85$) (51, 53). However, for artemisin, concentrations 30 minutes after oral intake were excluded from data analysis because levels were high and reflected probable saliva contamination by oral administration (53). This concern might have been addressed if saliva collection had been preceded by mouth rinsing, as described in another PK study of anticonvulsants in children (63). A study with risperidone in children showed that plasma and saliva were only correlated for pre-dose samples, demonstrating that saliva could be useful in measuring risperidone troughs but not in characterizing a full concentration-time curve (52). Correlation with plasma might therefore not be consistent across the concentration range or dosing interval. Lastly, a PK study of levetiracetam in adults and children showed that saliva collection was impractical in critically ill subjects due to thick and coalesced saliva (65). Therefore, clinical condition might affect salivary flow and, consequently, the ability to use saliva for sampling.

Saliva sampling is a promising alternative for PK trials in infants and is acknowledged by the FDA as a method for minimizing risk in pediatric PK trials (42). Because saliva drug concentrations may vary depending on several factors, however, a correlation with plasma PK should first be established. It is also worth noting that collection procedures should also be standardized to limit the impact of potential confounders, such as saliva flow rate and drug contamination, for oral drugs.

Exhaled breath

Drug diffusion of certain drugs across the alveolocapillary membrane allows measurement of drug concentration in exhaled breath. Drug concentration can be assessed in a gas matrix by ion-molecule reaction mass spectrometry (54). Advantages include high sensitivity and the possibility of real-time monitoring of drug concentration (54, 66). However, this method is highly dependent on the drug's ability to diffuse in the alveolar space, and not all drugs do that in measurable quantities. In addition, this type of sampling requires that participants are intubated, further limiting the applicability of exhaled breath measurements. The anesthetic agent propofol has been successfully measured in exhaled breath of adults via an endotracheal tube (54). End-expiratory propofol concentration was highly correlated with whole-blood concentration in real-time monitoring of anesthetized adults ($r=0.784-0.985$ Pearson) (54). One study in 8 children undergoing anesthesia showed a high correlation between plasma and end-expiratory propofol concentrations across the target concentration range ($r=0.98$; $p<0.01$). (67) There are no studies using exhaled breath measurements in infants.

5. Microdosing

Microdosing studies consist of evaluating PK after the administration of sub-pharmacologic doses, defined as $1/100^{\text{th}}$ of the dose of a test substance calculated to yield a pharmacologic effect with a maximum dose of $100\mu\text{g}$ (68). When the study drug is radiolabeled, it can be quantified in plasma samples using accelerator mass spectrometry, allowing characterization of the concentration-time profile and the generation of exploratory PK data (69). Recent

advances in analytical methods have also allowed quantification of non-radiolabeled compounds with the use of highly sensitive LC-MS/MS techniques (70). Advantages of this method include the administration of low doses with no expected therapeutic or toxicological effects. Moreover, low sample volumes are required to measure plasma concentrations (~250 µL per sample). When radiolabeled compounds are used, the amount of radioactivity exposure is considered harmless for adults, but safety in infants needs further evaluation. Lastly, a concern in microdose studies is that PK between microdoses and therapeutic doses might not be linear for some drugs because of saturation of metabolic pathways and transporters.

In adults, the success of microdosing PK is compound dependent. For some radiolabeled compounds such as acetaminophen (71), saxagliptin [71], and dapagliflozin (72) and non-radiolabeled compounds such as fexofenadine (70), microdosing PK estimates showed good agreement with PK estimates generated from standard dosing PK trials. In contrast, a microdosing study of warfarin showed differences in distribution between microdoses and therapeutic doses that were attributed to saturable tissue binding (73). Experience in infants is extremely limited with one abstract describing the administration of escalating dosing of ¹⁴C ursodiol to 5 infants (8; 26 and 80ng) (74). The microdose ursodiol concentration-time profile was successfully determined and PK parameters estimated, but no validation with therapeutic dose PK estimates was performed. In this study, radiological exposure was considered negligible though actual exposure was not reported.

The application of microdosing in infants is still very limited but could become useful, especially with the possible use of non-radiolabeled compounds. For the moment, regulatory agencies support its use in exploratory studies with no therapeutic intent (68, 75).

Conclusion

Appropriate dosing of drugs commonly used in infants is an urgent, unmet public health need and will decrease drug-related toxicity and improve therapeutic efficacy in this vulnerable population. Although PK trials are challenging in infants, innovative methods in PK sampling and analyses have been developed to overcome the obstacles encountered in conventional PK trials. Some of these methods, such as population PK modeling, are now well established and encouraged by regulatory agencies. Other more recent alternatives, like DBS sampling, opportunistic design, the use of non-blood matrices and PBPK, are promising strategies. However, validation and use of PBPK models in regulatory submissions are still lacking. Increasing experience with these novel methods will provide a balanced understanding of their strengths and limitations and will help improve the design of future studies, tailoring the right design to the right drug and population.

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Table 1
Pharmacokinetics studies in infants using minimal-risk study design

	Methods							Number of infants enrolled
	Microanalytical assays		PK Modeling and simulation		Opportunistic design		Alternative biological matrices	
	Ultra-low-volume drug assays	DBS	Sparse sampling*/Population PK	PBPK**	Drug administered per standard of care	Scavenged sampling		
Acyclovir(76)			x					79
Amikacin(77-80)			x			x		432
Amoxicillin(81)			x			x		190
Caffeine(17)		x					x	140
Caffeine(50, 82)			x					135
Cefepime(83)			x			x		41
Ciprofloxacin(84)			x					88
Cisapride(85, 86)			x					231
Clonidine(87)			x			x		36
Digoxin(88, 89)			x				x	223
Domperidone(90)			x					32
Emtricitabine(91)			x					32
Flucloxacillin(92)			x				x	55
Fluconazole(48)						x	x	55
Gentamicin(93-101)			x			x		1484
Ibuprofen(102, 103)			x					128
Indomethacine(104)			x					90
Ketorolac(105)			x					12
Levocetirizine(106, 107)			x					343 [§]
Meropenem(108)			x					37
Meropenem(8)	x		x			x		200
Metronidazole(64)						x		1
Metronidazole(10)	x		x			x	x	32

	Methods							Number of infants enrolled
	Microanalytical assays		PK Modeling and simulation		Opportunistic design		Alternative biological matrices	
Ultra-low-volume drug assays	DBS	Sparse sampling*/Population PK	PBPK**	Drug administered per standard of care	Scavenged sampling			
Metronidazole(19, 20)		x			x		56	
Micafungin(109)			x				47	
Midazolam(110, 111)			x			x	196	
Milrinone(112)			x				217	
Netilmicin(113)			x		x		74	
Nevirapine(114)			x				30	
Oseltamivir(45)					x		20	
Phenobarbital(115-117)			x		x		142	
Phenytoin(118)			x				83	
Piperacillin(9)	x				x	x	56	
Ropivacaine(119)			x				35	
Tenofovir(120)			x				32	
Theophylline(121, 122)			x		x		189	
Tobramycin(123, 124)			x		x		610	
Vancomycin(125-128)			x			x	555	
Zidovudine(129)			x				35	

DBS, dried blood spots; PBPK, physiologically based pharmacokinetics

^{*} 3 pharmacokinetic samples per subject

^{**} with prospective validation

[§] Include children >2y