C. Procedure for SPREC coding[UJ1][UJ2]

The SPREC parameters are fairly embedded with the H3Africa harmonized SOPs (hyperlink to SOPs) and these should be extracted. Each biospecimen will be assigned an eight (seven)-element-long code (H3Africa Consortium modified) that corresponds to seven pre-analytical variables for fluids(3). For complex derivatives a five-element-long code has been proposed. The SPREC will be applied to primary samples (e.g., whole blood, urine, and solid tissue), their simple derivatives (e.g., after centrifugation of collection tubes or mechanical disruption of tissues) and complex derivatives (e.g. DNA, RNA and cell lines). Primary samples are defined as biospecimens directly collected from the donor. Simple derivatives are defined as the samples prepared through a simple laboratory manipulation without the addition of chemical substances by the laboratory technician and without cell disruption or cell selection as part of a multistep process.[UJ3]Complex derivatives (e.g., nucleic acids, proteins, lipids, sorted cells, cultured cells, immortalized cells) are biospecimen derivatives whose isolation requires usage of multiple steps and/or addition of chemical substances including kits or in-house methods (these are not covered by SPREC and modifications have been suggested to capture them). The current modifications cover nucleic acids as most of the H3Africa researchers will be processing them.

The code letters are in a defined order, separated by sevenor four hyphens. The code letters are defined arbitrarilyor make use of the existing Laboratory Data Management System (LDMS) codes for the sample types and the primary container types. SPREC includes the sample type, collection type and a range of sample processing types constituting the entire preanalytical chain (for the case of H3Africa ends with submission to the Biorepositories). If the preanalytical option used is unknown or inconstant, the letter “X” is used. If the preanalytical option used is known but does not correspond to any of the standard options, the letter “Z” is used(3). The code will be linked to all aliquots of the corresponding sample held at the local collection centers.

The seven code element for simple biospecimensis defined below:

First code element: biospecimen type

Second code element: type of primary container

Third code element: precentrifugation

Fourth code element: centrifugation

Fifth code element: second centrifugation

Sixth code element: postcentrifugation

Seventh code element: storage condition

Eight code element: (H3Africa modification): no. of freeze-thaw cycles

The eighth code element has been included since the samples are expected to undergo repeated freeze-thaw cycles at the local collection sites as well as at biorepositories and thus this parameter becomes of importance for future sample use.

Table 1.Preanalytical variables included in the SPREC, modified version SPREC-01, applied to fluid samples

Type of sample	**SPREC**
Blood (whole)	BLD
Buccal cells	BUC
Unficolled buffy coat, viable	BUF
Ficoll mononuclear cells, viable	CEL
Fresh cells from non blood specimen type	CEN
24-h urine	U24
Urine	URN
Ficoll mononuclear cells, nonviable	PEL
Plasma, single spun	PL1
Plasma, double spun	PL2
Saliva	SAL
Serum	SER
Sputum	SPT
Other	ZZZ

Type of primary container	**SPREC**
Vacutainer acid citrate dextrose or equivalent	ACD
Vacutainer sodium EDTA or equivalent	SED
Vacutainer lithium heparin or equivalent	HEP
Vacutainerhirudin or equivalent	HIR
Oragene collection container or equivalent	ORG
Paxgene blood RNA+	PAX
Vacutainer potassium EDTA or equivalent	PED
Protease inhibitors	PIX
Polypropylene tube sterile	PPS
Paxgene blood DNA	PXD
Paxgene bone marrow RNA	PXR
Vacutainer sodium citrate or equivalent	SCI
S8820 protease inhibitor tablets or equivalent	PI1
Vacutainer citrate phosphate dextrose or equivalent	CPD
Vacutainer sodium fluoride/potassium oxalate or equivalent	SPO

Precentrifugation (delay between collection and processing)		**SPREC**
RT*	<2 h	A
3°C to 7°C	<2 h	B
RT	2-4 h	C

Centrifugation		**SPREC**
RT 10 min	<3,000 g no braking	A
RT 10 min	<3,000 g with braking	B
3°C to 7°C 10 min	<3,000 g no braking	C
3°C to 7°C 10 min	<3,000 g with braking	D
RT 10 min	3,000-6,000 g with braking	E
3°C to 7°C 10 min	3,000-6,000 g with braking	F
RT 10 min	6,000-10,000 g with braking	G
3°C to 7°C 10 min	6,000-10,000 g with braking	H
RT 10 min	>10,000 g with braking	I
3°C to 7°C 10 min	>10,000 g with braking	J
No centrifugation		N
Unknown		X
Other		Z

Second centrifugation		**SPREC**
RT 10 min	<3,000 g no braking	A
RT 10 min	<3,000 g with braking	B
3°C to 7°C 10 min	<3,000 g no braking	C
3°C to 7°C 10 min	<3,000 g with braking	D
RT 10 min	3,000-6,000 g with braking	E
3°C to 7°C 10 min	3,000-6,000 g with braking	F
RT 10 min	6,000-10,000g with braking	G
3-7°C 10 min	6,000-10,000g with braking	H
RT 10 min	>10,000 g with braking	I
3°C to 7°C 10 min	>10,000 g with braking	J
No second centrifugation		N
Unknown		X
Other		Z

Postcentrifugation delay		**SPREC**
<1 h	3°C to 7°C	A
<1 h	RT	B
1-2 h	3°C to 7°C	C
1-2 h	RT	D
2-8 h	3°C to 7°C	E
2-8 h	RT	F

3°C to 7°C	2-4 h	D
RT	4-8 h	E
3°C to 7°C	4-8 h	F
RT	8-12 h	G
3°C to 7°C	8-12 h	H
RT	12-24 h	I
3°C to 7°C	12-24 h	J
RT	24-48 h	K
3°C to 7°C	24-48 h	L
RT	>48 h	M
3°C to 7°C	>48 h	N
35°C to 38°C	<2 h	O
Unknown		X
Other		Z

Number of free-thaw cycles	**SPREC**
0	A
1 to 10	B
11 to 20	C
21 to 30	D
31 to 40	E
41 to 50	
>50	

8-24 h	3°C to 7°C	G
8-24 h	RT	H
>24 h	3°C to 7°C	I
>24 h	RT	J
Unknown		X
Other		Z

Long-term storage		**SPREC**
PP tube 0.5-2 mL[†]	-85°C to -60°C	A
PP tube 0.5-2 mL	-35°C to -18°C	B
Cryotube 1-2 mL	Liquid nitrogen[‡]	C
Cryotube 1-2 mL	-85°C to -60°C	D
Cryotube 1-2 mL	Programmable freezing to <-135°C	E
Straw	Liquid nitrogen	F
Straw	-85°C to -60°C	G
Straw	-35°C to -18°C	H
Straw	Programmable freezing to <-135°C	I
PP tube ≥5mL	-85°C to -60°C	J
PP tube ≥5mL	-35°C to -18°C	K
Microplate	-85°C to -60°C	L
Microplate	-35°C to -18°C	M
Paraffin block	RT	P
G		X
Other		Z

*RT, room temperature: 18°C to 25°C; [†]PP, polypropylene; [‡]Liquid nitrogen refers to either vapor or liquid phase.

Complex Biospecimens

The five code element for complex derivatives is defined below:

First code element: biospecimen type

Second code element: primary sample type

Third code element: extraction method

Fourth code element: storage condition

Fifth code element: no. of freeze-thaw cycles

Table 2.Preanalytical variables proposed for SPREC coding for complex variables

Type of sample	**SPREC**
RNA	RNA
DNA	DNA
Cell line	CLE
Proteins	PRN
Lipids	LPD
Sorted cells	SOC
Culturedcells	CCS

Primary sample type	**SPREC**

Storage conditions		**SPREC**
PP tube 0.5-2 mL[†]	-85°C to -60°C	A
PP tube 0.5-2 mL	-35°C to -18°C	B
Cryotube 1-2 mL	Liquid nitrogen[‡]	C
Cryotube 1-2 mL	-85°C to -60°C	D
Cryotube 1-2 mL	Programmable freezing to <-135°C	E
Straw	Liquid nitrogen	F
Straw	-85°C to -60°C	G
Straw	-35°C to -18°C	H
Straw	Programmable freezing	I

Blood (whole)	BLD
Buccal cells	BUC
Unficolled buffy coat, viable	BUF
Ficoll mononuclear cells, viable	CEL
Fresh cells from non blood specimen type	CEN
Ficoll mononuclear cells, nonviable	PEL
Plasma	PL
Saliva	SAL
Serum	SER
Sputum	SPT
Other	ZZZ

Extraction method	**SPREC**
Automated Kit-based	A
Automated In-house	B
Manual Kit-based	C
Manual In-house	D

	to <-135°C	
Microplate	-85°C to -60°C	K
Microplate	-35°C to -18°C	L
Unknown		X
Other		Z

Number of free-thaw cycles	**SPREC**
0	A
1 to 10	B
11 to 20	C
21 to 30	D
31 to 40	E
41 to 50	F
>50	G

*RT, room temperature: 18°C to 25°C; †PP, polypropylene; ‡Liquid nitrogen refers to either vapor or liquid phase.

For example:

1. A Serum biospecimen**SER-SST-A-E-N-A-G-A**: This corresponds to a serum (**SER**) biospecimen that has been collected from a serum collection tube (**SST**), whose precentrifugation delay is <2 hours at room temperature (**A**); centrifugation has been done at ambient temperature at 3,000 to 6,000 g with braking (**E**). Only one centrifugation step was done (**N**) and the delay between centrifugation and freezing was <1 hour at 3°C to 7°C (**A**). Serum was stored in straws at a temperature between -85°C and -60°C (**G**) and has had no freeze-thaw cycle (**A**).
2. DNA biospecimen **DNA-BLD-A-A-A**: This corresponds to a DNA (**DNA**) biospecimen derived from blood (**BLD**), by automated method using a kit (**A**) stored in a 0.5-2 mL PP tube at -85°C to -60°C (**A**)and has undergone no freeze-thaw cycle (**A**).