PASC in Pregnancy and Postpartum Registry | Biospecimens Recommended Measures

Tier 1 Biospecimens (Neonate)				
Specimen	**Collection**	**Timing**	**Storage**	**Sample Analyses**
Neonate/Cord Blood	• **Plasma / Buffy Coat:** EDTA tubes (at least 10mL total) • **Serum:** Serum separator (5mL)	• Date of Draw	• **Plasma:** Freeze in 200 µL (max 1 mL) aliquots, store at -80°C • **Serum:** Freeze in200 µL (max 1 mL) aliquots, store at -80°C • **Buffy coat:** Freeze, store at -80°C	**Plasma / Serum:** • SARS-CoV-2 RNA viral load • SARS-CoV-2 antibodies (IgG, IgA, neutralizing antibody evaluation) • Cytokine analysis **Buffy coat:** Analysis of cellular fraction (Evaluation of properties of specific cellular fractions such as T-cells, monocytes)
Neonatal Respiratory Specimens	• Nasopharyngeal swab (preferred) • Nasal swab • Oropharyngeal swab (RTq-PCR) • Saliva vial / cup	• Delivery • At time of acute illness (if applicable)[18]	• **Swab:** Swirl in PBS, aliquot PBS and freeze at -80°C • *If diagnostic swab can be retrieved from clinical lab, it can be used for quantitative (viral load) and other assays* **Saliva:** Process with DTT, store at -80C	• Use to confirm SARS-CoV-2 negative status in control group • SARS-CoV-2 RNA viral load

Tier 1 Biospecimens (Maternal)				
Specimen	**Collection**	**Timing**	**Storage**	**Sample Analyses**
Maternal Blood[19]	• **Plasma / Buffy Coat:** EDTA tubes (at least 10mL total) • **Serum:** Serum separator (5mL)	• At time of acute illness *and / or* • At delivery	• **Plasma:** Freeze in 200 µL (max 1 mL) aliquots, store at -80°C • **Serum:** Freeze in200 µL (max 1 mL) aliquots, store at -80°C • **Buffy coat:** Freeze, store at -80°C	**Plasma / Serum:** • SARS-CoV-2 RNA viral load • SARS-CoV-2 antibodies (IgG, IgA, neutralizing antibody evaluation) • Cytokine analysis **Buffy coat:** • Analysis of cellular fraction (Evaluation of properties of specific cellular fractions such as T-cells, monocytes)
Cord Blood	• **Plasma / Buffy Coat:** EDTA tubes (at least 10-15 mL total, may be less for pre-term deliveries) • **Serum:** Serum separator (7.5mL)	• At delivery	• **Plasma:** Freeze in 200 µL (max 1 mL) aliquots, store at -80°C • **Serum:** Freeze in 200 µL (max 1 mL) aliquots, store at -80°C • **Buffy coat:** Freeze, store at -80°C	**Plasma / Serum:** • SARS-CoV-2 RNA viral load • SARS-CoV-2 antibodies (IgG, IgA, neutralizing antibody evaluation) • Cytokine analysis **Buffy coat:** • Analysis of cellular fraction (Evaluation of properties of specific cellular fractions such as T-cells, monocytes)
Placenta	• Fixed tissue	• At delivery	• Store at 4°C until fixation • Once fixed, can be stored as formalin-fixed paraffin embedded blocks	• RNA *in situ* hybridization (RNA-ISH)
	• Maternal side biopsy *and* • Fetal side biopsy	• As soon as possible; within 1-2 hours of delivery maximum (RNA will degrade)	• Process in RNA later, following manufacturer instructions • Store preserved tissue at -80°C (or -20°C if 80°C not available)	• SARS-CoV-2 RNA analyses[20]
Colostrum and/or mature milk	• Pump into colostrum cup *or* • Hand expression into colostrum cup For larger volume: • Pump into pumping containers or storage containers	• During delivery admission *or* • At postpartum visit	• **Small volume:** Aliquot (e.g. 1 mL aliquots) and store at –80°C • **Large volume** (e.g. 10 mL or above): Spin and separate cellular fraction and supernatant. Aliquot (e.g. 1 mL aliquots) and store at –80°C	• SARS-CoV-2 RNA viral load[21] • SARS-CoV-2 antibodies (IgG, IgA, neutralizing antibody evaluation)

Tier 2 Expanded Specimen Collection (Maternal)				
Specimen	**Collection**	**Timing**	**Storage**	**Sample Analyses**
Maternal Respiratory Specimens[22]	• Nasopharyngeal swab (preferred) • Nasal swab	• 11-14 weeks[23] • 18-22 weeks[5] • 28-32 weeks[5]	• **Swab:** Swirl in PBS, aliquot PBS and freeze at -80°C	• Use to confirm SARS-CoV-2 negative status in control group

	• Oropharyngeal swab (RTq-PCR) • Saliva vial / cup	• Delivery • At time of acute illness (if applicable)[24]	• *If diagnostic swab can be retrieved from clinical lab, it can be used for quantitative (viral load) and other assays* • **Saliva:** Process with DTT, store at -80C	• SARS-CoV-2 RNA viral load
Maternal Blood	• **Plasma, Buffy Coat, and/or PBMC:** EDTA tubes (at least 10mL total) • **Serum:** Serum separator (5mL)	• 11-14 weeks[5] • 18-22 weeks[5] • 28-32 weeks[5] • Delivery • At time of acute illness	• **Plasma:** Freeze in 200 µL (max 1 mL) aliquots, store at -80^{o}C • **Serum:** Freeze in 200 µL (max 1 mL) aliquots, store at -80^{o}C • **Buffy coat:** Freeze, store at -80^{o}C • **PBMC:** Store in freezing media in liquid nitrogen (LN_2)	• Inflammatory markers (e.g. IL-6, TNF-a, IL-1B, IFN-g, IL-10, CRP/ESR) • T-cell, monocyte, other specific cell fraction experiments (EDTA only) • AM cortisol or CRH
	• PaxGene tube (2.5 or 5 mL)	• 11-14 weeks[5] • 18-22 weeks[5] • 28-32 weeks[5] • Delivery • At time of acute illness	• Shake vigorously • May keep at room temp for 2-24 hrs • Freeze whole tube, store at -80^{o}C	• Transcriptomic/global gene expression analyses
Cord Blood	• **For PBMC:** EDTA tubes (at least 10-15 mL total)	• At delivery	• **Isolated PBMC:** Store in liquid nitrogen (LN_2)	**PBMC:** • Analysis of cellular fraction (scRNA-Seq, evaluation of properties of specific cellular fractions such as T-cells, monocytes) • Genotyping (array-based)
	• PaxGene tube (2.5 or 5 mL)	• At delivery	• Shake vigorously • Keep at room temp for 2-24 hrs • Freeze whole, store at -80^{o}C	• Transcriptomic/global gene expression analyses
Placenta	• Maternal side placental biopsy *and* • Fetal side placental biopsy	• As soon as possible; within 1-2 hours of delivery maximum (RNA will degrade)	• Process in RNA later, following manufacturer instructions • Store preserved tissue at -80^{o}C	• SARS-CoV-2 RNA viral load • RNA RTq-PCR for specific genes of interest • DNA methylation analyses • Genotyping (array-based)
	• Full thickness biopsies *or* • Remaining whole placenta	• Take biopsies in pathology lab	• Formalin-fixed, paraffin-embedded blocks	• RNA *in situ* hybridization (RNA-ISH) to define placental infection

[1] If a participant that was previously designated as a control becomes ill with COVID-19, specimens collected after that point can no longer be used as a control

[19] Blood draws for research in pregnancy should not exceed 50 mL in 8 weeks. Those with Hct < 24 should not provide blood for research

[20] Preservation in RNA later permits both RNA and DNA analyses

[21] Specific guidance to participants and adherence to breast cleaning protocols is critically important if breastmilk viral load quantification is planned

[22] Respiratory specimens only necessary to be collected to document COVID-19 negative status at the time of collection if the participant provides other samples for study

[23] Can alternatively collect at 1st, 2nd, 3rd trimester appointments. Ideally, align maternal respiratory specimens collection with maternal blood draw

[24] If a participant that was previously designated as a control becomes ill with COVID-19, specimens collected after that point can no longer be used as a control

			• Fixed tissue can be sectioned and stored on slides and in in paraffin-embedded blocks	• ACE2/TMPRSS2 protein expression patterns • Cd68/CD163 for Hofbauer cell hyperplasia and chronic histiocytic intervillositis
Breastmilk	**Large volume:** • Pump into pumping containers or storage containers	• At delivery *and/or* • Post-partum	• **Large volume** (e.g. 10 mL or above): Spin and separate cellular fraction and supernatant	**Cellular fraction of breastmilk:** • Sequencing • Isolation of T-cells, NK cells, and antibody-producing B cells

Tier 3 (Maternal)				
Specimen	**Collection Method**	**Timing**	**Storage**	**Sample Analyses**
Saliva (Tier 3)	• Oragene	• Follow time restrictions included in instructions	• Process with DTT • Store at -80^{o}C	• Genotyping
Placenta	• Maternal biopsy *and* • Fetal side biopsy	• As soon as possible; within 1-2 hours of delivery maximum (RNA will degrade)	• Snap freeze tissue in liquid nitrogen (preferred) or on dry ice • Store at -80^{o}C	• Protein isolation • Single-cell RNA-seq • DNA/RNA extraction
	• Full thickness biopsies *or* • Remaining whole placenta	• Take biopsies in pathology lab	• Formalin-fixed, paraffin-embedded blocks • Fixed tissue can be sectioned and stored on slides and in in paraffin-embedded blocks	• CD147 & CD26 protein expression patterns
	• Membrane or decidua basalis	• As soon as possible • Within 1-2 hours of delivery maximum (RNA will degrade)	• Process fresh for cell isolation per protocol	• Inflammatory/immune analyses (e.g., FACS, flow cytometry, transcriptomics, pro-inflammatory cytokine quantification)