# Media Extraction for LC-MS
# Llinás Lab 2016 (Updated 02/22/2017)

**Summary:** This procedure is tailored to measure the exchange of metabolites between parasites and the environment in the absence of media changes. This can be performed at a single time point or as a time course experiment. It is well suited for comparing parental and knockout/knockdown lines or drug treated parasites.

## Materials:

1) Synchronized cultures of ring-stage parasites – 0-3 hours post invasion at ~1-3% parasitemia in 2% hematocrit
2) Microcentrifuge, chilled to 4°C
3) One bucket of ice
4) Ice-cold 100% Methanol (HPLC-grade) with isotope labeled compound (see Note #3 below).
5) Vortex

## Experimental Setup:

**Note: Parasite cultures should be mycoplasma free before initiating your experiment.**

1) Parasites should be synchronous and staged accordingly. Data from mixed stage infections or improperly staged parasite lines will be difficult to interpret because of the varied metabolic capacities of the IDC stages. Additionally, the experiment should utilize an uninfected control since the amount of uninfected cells present during an experiment of this nature typically largely outweighs that of the infected cells. This allows for the effectively removal of any uninfected metabolite signal and/or to account for alterations to the host cell.
   **Note**: An uninfected red blood cell control is always good to have, but is more important for drug treatment assays to account for host cell metabolic changes.
2) Once the parasites are synchronous they should be split to a relatively low parasitemia ~1-3% rings for the start of the experiment. High parasitemia should be avoided as glucose availability becomes the limiting factor and you want to avoid measuring dead or stalling parasites. Similarly, very low parasitemia should be avoided as it reduces the amplitude of changes that will be measurable over time.
3) Once split, a sample of the fresh media should be taken to mark time zero. Typically this is done in triplicate to reduce both technical variability and systematic variability in the instrument.
4) Depending on the severity or stage of phenotype, samples are taken periodically (every X minutes or hours).
   **Note**: Samples should only be collected within a single IDC as hemolysis from merozoite release complicates further metabolic interpretation.

## Metabolite Extraction:

1) Simply, 100µL of media is transferred to a 1.6mL microcentrifuge tube, on ice, containing 900µL of ice cold 100% HPLC grade methanol, for a final concentration of 90% methanol.
   **Note:** It is recommended to spike-in $^{13}C_4$, $^{15}N$-Aspartate (Cambridge Isotope, Cat No. CNLM-544-H-PK) or a similar isotopically labeled compound (final concentration 0.5 µM) as an internal standard for LC/MS analysis.
   a. If there is the possibility of cellular contamination (parasite or erythrocyte), the 100µL sample should be centrifuged briefly @ 8,500 RPM (~30sec) to clarify before transferring the supernatant to the ice-cold methanol.
2) Vortex the samples for 10 seconds and store in the -80°C freezer until ready to dry-down under nitrogen gas (preferred method) or lyophilize using a speed-vac.
   **Note**: While samples can be store in methanol at -80°C for several weeks, they are more stable if immediately dried down under nitrogen gas and stored as a dry metabolite "pellet" at -80°C. Once frozen, the samples should be shipped on dry ice and only thawed for LC/MS processing.

**If shipping to the Llinás lab: Please provide a complete list of sample IDs with cell counts and label all samples clearly. For LC/MS, dried extracts are resuspended in HPLC-grade water in a final volume of 100 µL.**