Enabling a Healthier World

Lonza

Primary Hepatocytes for Research

A Bench Guide for Drug Development Applications

Introduction

The handling and care of primary hepatocytes is critical for their optimal performance in *in vitro* research. This guide provides background information on applications and culture formats, with tips to help you achieve optimal results for your hepatocyte studies and to inspire confidence in your research. Lonza created this guide with all levels of hepatocyte users in mind — from student researchers to veteran R&D scientists.

Abbreviations Used in this Bench Guide

Abbreviations: ADME – absorption, distribution, metabolism, excretion; CL – clearance; CLint – intrinsic clearance; GST – glutathione S-transferase; HBV – hepatitis B virus; HCV – hepatitis C virus; iPSC – induced pluripotent stem cells; PHH – primary human hepatocytes; SULT – sulfotransferase; UGT – uridine 5'-diphospho-glucuronosyltransferase; NASH – Non-alcoholic steatohepatits

Why Hepatocytes?

Figure 1 shows the functions of the human liver. The liver plays a critical role in vertebrate biology. It has many important metabolic functions, including the regulation of glucose and cholesterol metabolism, the production of plasma proteins including clotting factors, and the detoxification of endogenous and exogenous compounds. The liver also produces various hormones involved in insulin regulation, blood pressure, and blood lipid levels.

Because of the many physiological processes that depend on the liver, a fundamental understanding of liver biology and the ability to address these at the benchtop is essential for researchers involved in creating new, life-saving medicines.

The liver is composed of five major cell types, and the hepatocyte is the most abundant cell type. Hepatocytes comprise approximately 70% of the liver cell population and are responsible for most metabolic and hormonal processes. The other four cell types, collectively known as

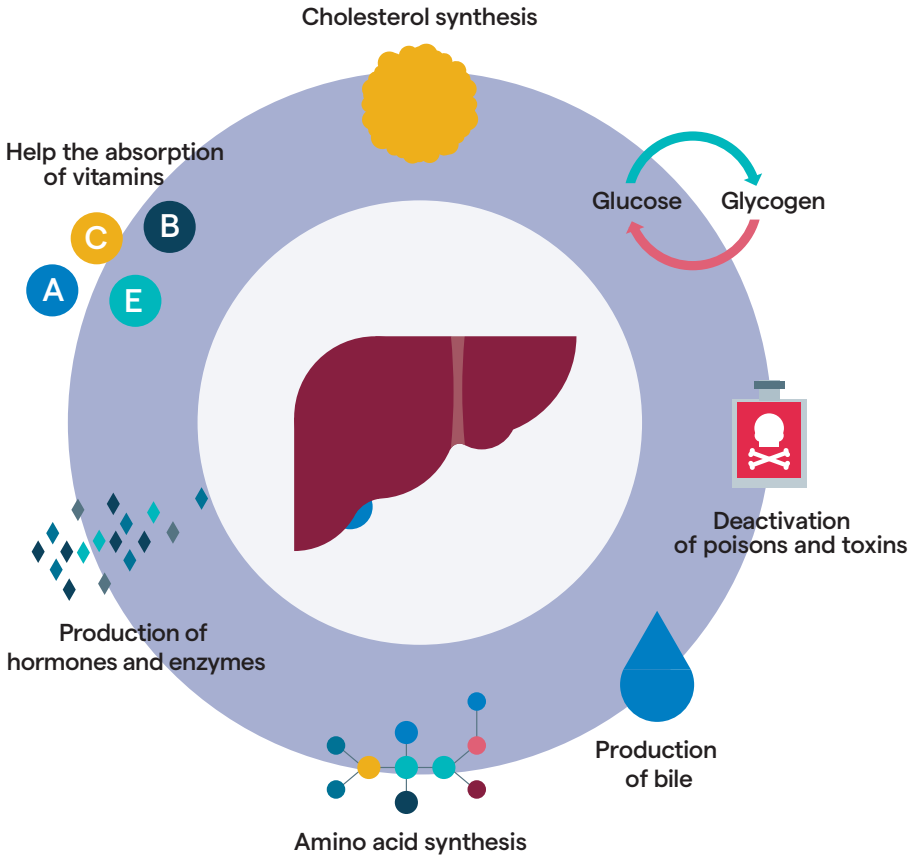


Figure 1.
The liver is the primary organ for xenobiotic metabolism and detoxification processes in vertebrates.

hepatic non-parenchymal cells, consist of resident macrophages called Kupffer cells, stellate cells, liver sinusoidal endothelial cells, and cholangiocytes. These cells serve to support the liver structure, transport molecules in and out, and communicate with the immune system.

One of the greatest challenges in drug development is the prediction of the safety and the metabolic fate of a new drug before it enters human clinical trials. Because the liver is the major site of metabolism and detoxification, *in vitro* cell systems that mimic the liver are significantly useful for predicting the consequences of drugs administered to humans in the clinic.

The ability to isolate highly pure hepatocyte populations from non-transplantable, donated human livers to mimic the human liver environment has therefore become an integral part of the drug development pipeline.

How are Primary Hepatocytes Different from Hepatic Cell Lines?

Hepatocytes directly isolated from liver tissue are called primary hepatocytes. Figure 2 is a schematic that shows the procedure for the isolation of primary human hepatocytes, and their cellular morphology in suspension and on plates.

Decades of research and development has resulted in a robust isolation process for primary hepatocytes, yielding cultures with purities over 98%. Once isolated from the tissue, the hepatocytes may be used right away or cryopreserved for later use. The functionality of primary hepatocytes in culture is very similar to that of *in vivo* hepatocytes, as indicated by albumin production, urea production, and a variety of metabolic enzyme activities[1-2].

Many other cell culture models can be used to simulate the liver environment for research and development. Some of these alternative hepatocyte models include tumor cell lines such as Huh-7 and HepG2 cells. Tumor cell lines include the advantage of self-renewal, which saves resources and enables the more rapid production of genetic models for mechanistic research. However, the metabolic activity of tumor lines is 10 – 1,000 orders of magnitude less than that of primary hepatocytes[3-4].

Another type of hepatocyte alternative is HepaRG® cells, a tumor cell line that can differentiate into cells that have a phenotype resembling that of primary hepatocytes. HepaRG® cells have gained widespread acceptance as a hepatocyte alternative, especially when primary hepatocyte availability is limited ⑦.

Other renewable hepatocyte-like alternatives include cells differentiated from induced pluripotent stem cells (iPSC-derived hepatocytes) and primary hepatocytes that have been genetically altered to proliferate to some degree, such as Upcyte® Hepatocytes and Corning® HepatoCells[5-6].

All of these alternatives, while useful for many niche applications, consistently fall short in functionality compared to primary hepatocytes.

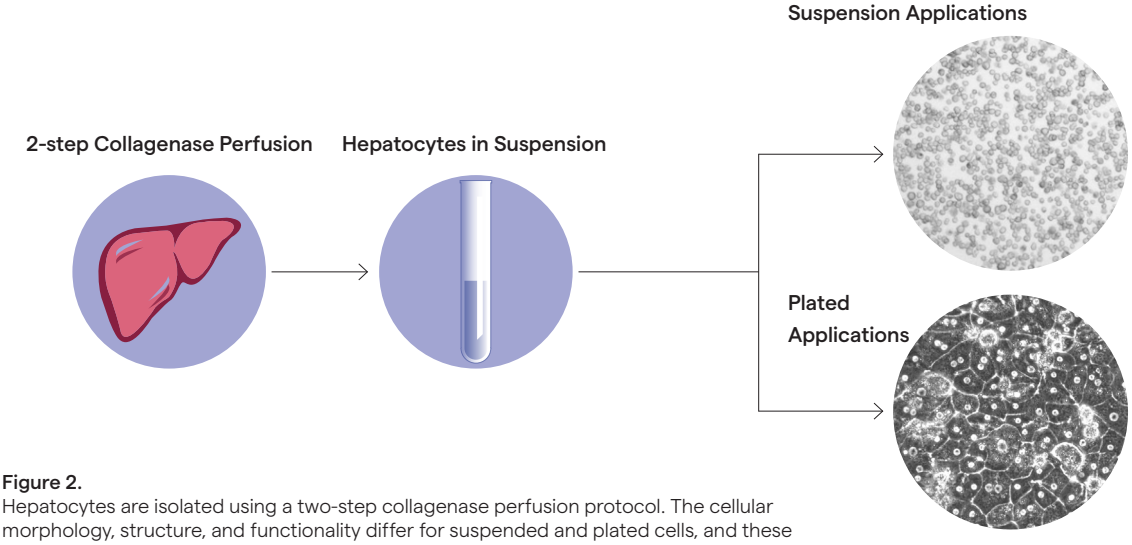


Figure 2.
Hepatocytes are isolated using a two-step collagenase perfusion protocol. The cellular morphology, structure, and functionality differ for suspended and plated cells, and these differences are important for specific applications of hepatocytes.

Applications of Hepatocytes in Cell Culture

The number of applications in drug discovery, development, and basic research using primary hepatocytes continues to expand (Figure 3). Whether hepatocytes should be used in suspension or plated formats or in 3D cultures depends on the specific application. In this section we outline the various formats for applications within the following research areas:

- Drug metabolism
- Drug and Chemical toxicity
- Drug Transporters
- Xenotransplantation/humanized animal models
- Disease research

Drug Metabolism Assays

Table 2. Metabolism Applications Summary

Application	Description	Hepatocyte Format
Metabolic Profiling	Determines the chemical class of a drug metabolite	Rapid Clearance[1]: Suspension Low Clearance: Plated
Metabolite Identification	Determines the specific molecular identity of a drug metabolite	Rapid Clearance: Suspension Low Clearance: Plated
Metabolic Stability	Determines the rate of transformation of a parent drug compound to metabolite(s)	Rapid Clearance: Suspension Low Clearance: Plated
Species Comparison	Compares the metabolic profile produced by human hepatocytes and multiple species of animal hepatocytes to determine which best correlates with human *in vivo* profiles	Rapid Clearance: Suspension Low Clearance: Plated
Drug Uptake	Determines the kinetics of drug transport into the cell	Rapid Clearance: Suspension Low Clearance: Plated
Enzyme Induction	Determines the risk of a drug-drug interaction between the compound of interest and other common drugs (or drug-metabolizing enzymes)	Plated
Drug Transport Kinetics	Determines the rate of accumulation and excretion of drugs on the basolateral membranes as well as the rate of biliary efflux	Plated or suspension

[1]Clearance refers to the rate at which a drug is cleared from the system.

Primary hepatocytes are a key reagent for understanding the metabolism of drugs and chemicals including identifying metabolites, tracking metabolic clearance, and determining species differences. Depending on the specific application, hepatocytes used in drug metabolism studies may be used in plated or suspension formats (Table 2)

Suspension Metabolism Assays

The metabolic activity of freshly isolated hepatocytes is most similar to that of the intact tissue. As a result, shortly after isolation, researchers can use hepatocytes still in suspension for short term metabolic assays.

It was formerly believed that only freshly isolated hepatocytes would reflect the appropriate metabolism, but advancements in cryopreservation techniques now allow metabolic reactions to proceed following the recovery of cryopreserved cells[1–2, 8]. Cryopreservation also allows researchers to prequalify functionality of hepatocytes from specific donors. By purchasing large numbers of vials from the same batch, researchers enable more streamlined and convenient use of hepatocytes in routine standardized studies. Cryopreservation was also key to overcoming the historical limited availability of hepatocytes.

Suspension metabolism assays include

- Metabolic profiling
- Metabolite identification
- Metabolic stability
- Cell uptake kinetics

For metabolism assays, hepatocytes are used in wells of a cell culture plate that are rotating on a platform to maintain cells in suspension. Drug is added to these wells and assays are performed over time to record metabolic activity of the cells towards the target drug. For transporter uptake kinetics studies, a common approach known as the oil-spin technique measures radiolabeled drug concentrations inside the cell following a very short incubation period. Non-radiolabel techniques using mass spectrometry can also be applied.

These routine metabolism and uptake assays using hepatocytes in suspension are typically applied to large numbers of molecules early in development to help prioritize chemicals for further development.

Pooled Donor Suspension Hepatocytes

Most often, suspension metabolic studies are performed using hepatocytes pooled from multiple donors. Pooling of donors results in greater confidence that the metabolites and the rate of metabolism observed are reflective of the general population rather than that of a single donor. Pools of 10–20 donors is optimal, but some pools with 50–100 donors can be produced. DonorPlex™ Hepatocytes from Lonza are provided as 10–20– and 50–donor pools from a mixture of genders or single genders.

Because robust metabolic activity is measured in hepatocyte both before and after pooling, its possible to predict the metabolic activity of a pool of single donors prior to pooling. This enables companies to have a continuous supply of hepatocytes with roughly the same activity levels. A custom DonorPlex™ Hepatocyte project can also provide donor pools with varying activity levels for project specific applications.

Plated Metabolism Assays

CYP induction potential

The most common application of plated hepatocytes for *in vitro* drug metabolism and pharmacokinetics (DMPK) studies is the determination of the induction of various CYP450 enzyme genes and activities. The amount and activity of these enzymes are known to increase when cells are exposed to certain chemical substances due to increased gene transcription of the CYP enzymes. An increase in the activity of a particular P450 enzyme might also affect a different drug in a patient's regime, leading to a drug-drug interaction (DDI) that could impact the efficacy and safety of more than one drug. Regulatory agencies require an analysis of the DDI potential in hepatocytes for specifically CYP3A4, CYP1A2, and CYP2B6 genes, results from which indicates whether a clinical DDI study is required[9].

Efflux transporter inhibition potential

A second major application of plated hepatocytes is the determination of the inhibition of the transporter-mediated efflux of a substance by a particular drug. When the hepatocytes are plated onto collagen and then overlaid with more collagen or another basement membrane extract such as the Corning® Matrigel® Matrix, their polarized cell surface structures form as they are maintained *in vivo*. This creates a small bile pocket in which the bile efflux transporters are re-expressed at the cell surface. The consequences of the inhibition of bile efflux transport range from drug-drug interactions to overt liver toxicity due to the buildup of intracellular bile acids.

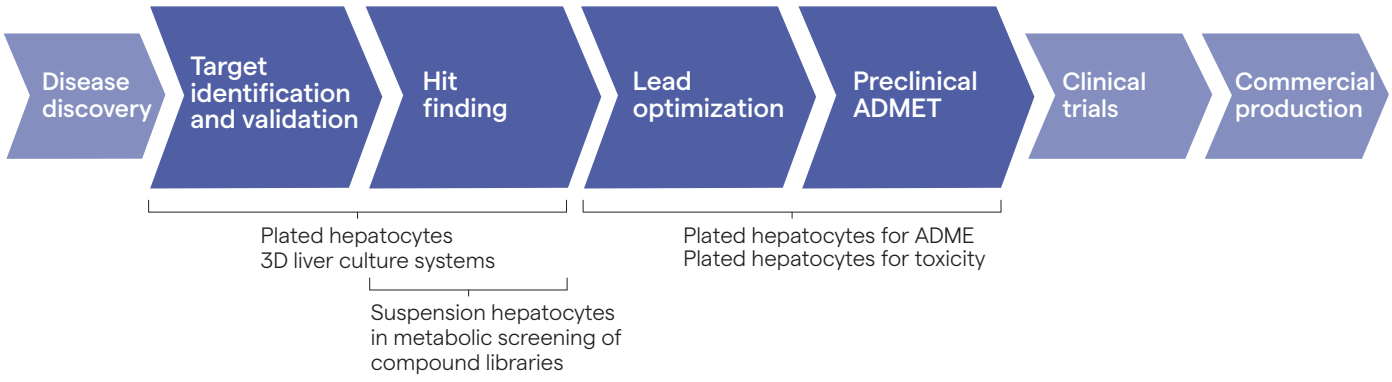


Figure 3.
Various hepatocyte formats can be applied at the different stages of the drug development pipeline.

Low turnover metabolism

Finally, the pharmaceutical discovery of drugs that are more slowly metabolized has become increasingly desirable. Slower drug metabolism translates into longer-lasting efficacious levels of a drug, so a patient can take the drug less frequently, which can increase compliance and decrease production costs. However, suspension hepatocytes are not viable or metabolically active long enough to allow the accurate measurement of the basic metabolic properties of such drugs. Since plated hepatocytes maintain their metabolic activity much longer than cells in suspension, researchers use them for assays that have a duration of 4 hours or longer.

One of the greatest challenges for assaying substances with low clearance rates using plated hepatocytes is that the media must be refreshed daily. If the media is not refreshed daily, the concentration of nutrients dwindles and metabolic waste builds up. When measuring metabolites that are produced slowly, changing the media can be detrimental. In such instances, other, more advanced models for hepatocyte culture may be used, such as co-culture models, 3D spheroids, or "organ-on-a-chip" microphysiological systems[10].

Table 3. Applications for Hepatotoxicity

Applications	Description	Hepatocyte Formats
Cell-based Toxicity	Short term assays to examine the acute effects of drugs and toxicants on cell health	Plated monolayer cultures 3D cultures/ Spheroids Co-Cultures
Mechanistic Toxicity	Short and long term assays to determine toxicity mechanisms. Results are often used to suggest structure-activity relationships and molecular signatures that indicate risk	Plated monolayer cultures3D cultures/ Spheroids Co-Cultures

Cellular Toxicity

Basic cellular toxicity studies are most frequently performed very early in the drug development pipeline (shown in Figure 3), using cell lines such as the HepG2 tumor cell line. Later in the drug development pipeline, the discovery of toxicity in animal models or phase I trials may trigger further investigation of toxic mechanisms that require more physiolgically relevant *in vitro* models. These investigations could include a comparison of different structures to further optimize a lead candidate and reduce the chances of clinical liver toxicity later in development. The type of mechanistic study selected in such cases is often highly dependent on the observations. Whereas common applications such as transcriptomics can be readily applied, investigative toxicologists are most often tasked with developing entirely new models to aid in the understanding and elimination of the toxic potential of a drug candidate. Primary hepatocytes in a monolayer are a good model for hepatocyte-specific toxicities, (Table 3).

Emergence of Spheroid Microtissues for Liver Toxicity

Liver toxicity often requires interactions between many cell types and enteroendocrine functions not readily modeled in cell culture. New models such as co-cultures with fibroblast cells, in both monolayer and 3D spheroid models, have been shown to increase the predictive power of cell culture models[11 – 12].

The simplest of these models is the self-assembled spheroid. Using an inexpensive commercially available low attachment, round-bottom, cell culture plate, hepatocytes will self-assemble into a spheroid shape. In the presence of serum containing medium, the spheroid forms a tight structure in about 4 – 8 days (Figure 4). The functionality of the hepatocytes in the spheroid is equivalent to that in sandwich culture in the early days, but when cultured longer, the spheroid doesn't rapidly de-differentiate and lose functionality. Cultures of hepatocyte spheroids with steady metabolic activity upwards of 30 days with clear advantages for toxicity testing have been reported[13 – 14].

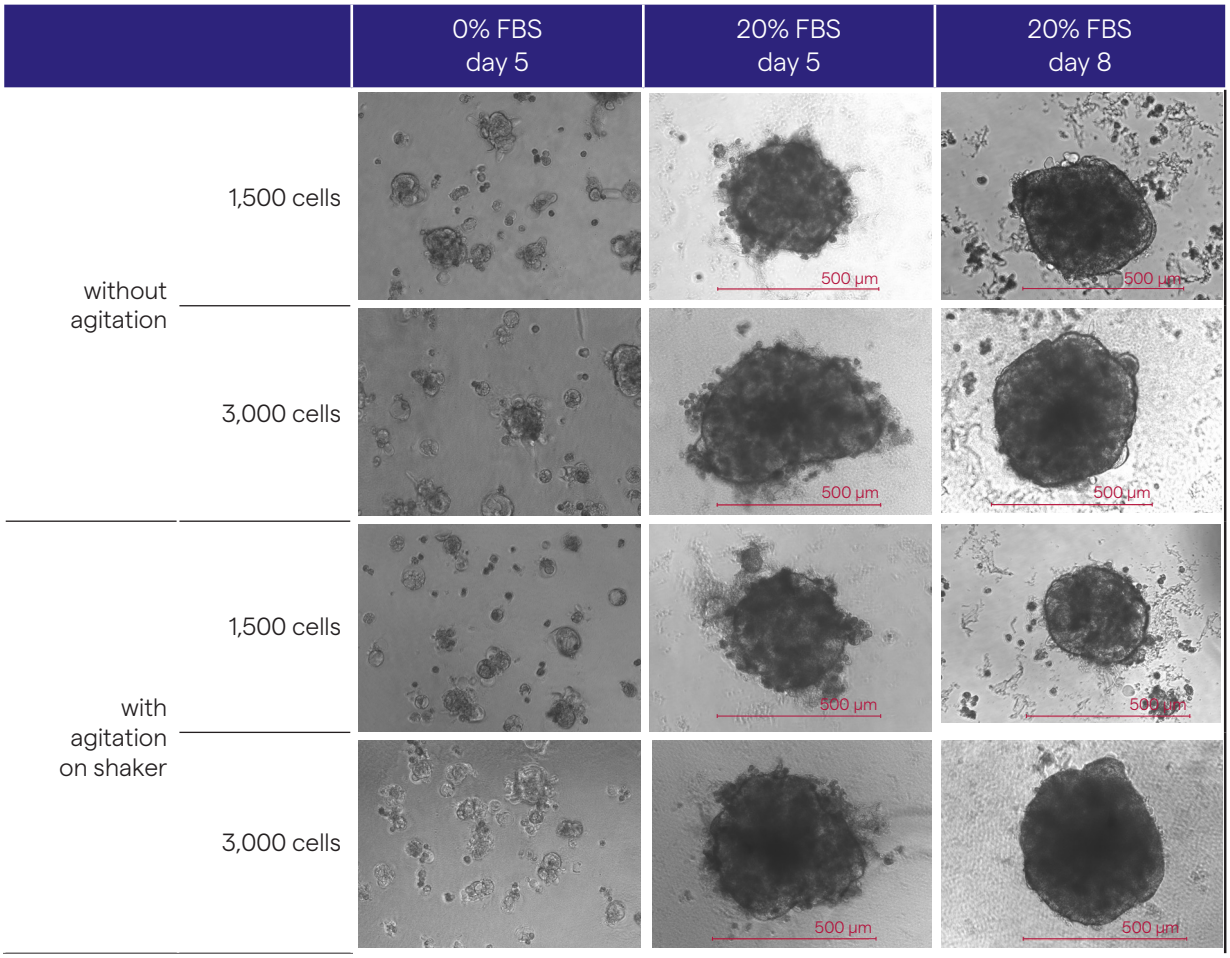


Figure 4.
Successful spheroid formation in presence of serum. 1,500 or 3,000 primary human hepatocytes were seeded in ULA U-bottom plates. Cells were left undisturbed for 5 days with or without gentle agitation.

Other Research Applications

There are many emerging applications for primary hepatocytes worth mentioning including the ability to create human liver tissue in mice through injecting human hepatocytes into the spleen (Table 4). Additional applications include modeling infectious diseases that either impact the liver directly, or use the liver for pathogen life cycle (Table 5), and metabolic diseases such as diabetes and NASH (Table 6)

Table 4. Humanized Mouse Models

Applications	Description	Hepatocyte Formats
Humanized mouse liver	A process where human hepatocytes are injected into mice with damaged livers in order to allow the liver to regenerate with human cells.	Highest quality plated monolayer cultures

Humanized Mouse Liver

An immunocompromised mouse model can be made to house a liver composed almost entirely of human liver cells. The purpose of creating such a model is to enable human-relevant liver pharmacokinetics and pharmacodynamics studies to be performed in an intact mouse model. By using a humanized mouse model, many species-species differences in metabolism between mouse and human can be eliminated as a variable in a determination of the value of a mouse model to predict human clinical outcomes. (15)

Table 5. Infectious Diseases.

Applications	Description	Hepatocyte Formats
Hepatitis infection model	A process in which primary human hepatocytes are infected with the hepatitis virus for drug screening or biomarker discovery/development	Highest quality plated monolayer cultures 3D cultures/Spheroids Co-Cultures
Malaria parasite infection model	A process in which primary human hepatocytes are infected with Plasmodium falciparium varieties for drug screening or biomarker discovery/development	Highest quality plated monolayer cultures 3D cultures/Spheroids Co-Cultures

Hepatitis

Many varieties of the hepatitis virus cause inflammation of the liver, which can lead to severe illness or even death when left untreated. Millions of people are infected with hepatitis viruses worldwide and it remains a leading cause of hepatocellular carcinomas, especially in individuals born in the U.S.A. between 1945 and 1965. While cures have been developed for the deadliest variant, Hepatitis C (HCV), reinfection can occur because HCV is very prone to mutation. This tendency to mutate has also hindered the development of a HCV vaccine.

While an effective vaccine is available for the more common Hepatitis B (HBV) virus, it still infects millions annually and causes illnesses ranging from flu-like symptoms to liver failure. HBV is a DNA virus that utilizes the nuclear machinery of the hepatocyte to replicate. Infection and subsequent replication can be modeled in primary human hepatocytes in culture, and has been used to develop large scale screening protocols for new drugs that attack the progression of the virions to the closed circular DNA, which eventually leads to inflammation and other symptoms. So far, HBV has eluded efforts to develop curative drugs[16 – 17].

Malaria

Nearly half of the world's population lives in regions where inhabitants are at risk for malaria infection and more than 500,000 people worldwide die from the disease each year. Malaria is caused by a Plasmodium parasite, which has a life cycle that includes the human liver; specifically, the infection of hepatocytes. As shown in Figure 4, Plasmodium sporozoites undergo a major replication event in the hepatocyte before entering the next stage of the life cycle in the blood. Once the parasite is in the blood, the infected person begins to experience the symptoms associated with malaria; alternating chills and fever, fatigue, and others. The development of clinical interventions for the liver stage of the Plasmodium life cycle has become of interest, not only to prevent malaria symptoms, but also the spread of the parasite back to the mosquito, through the blood.

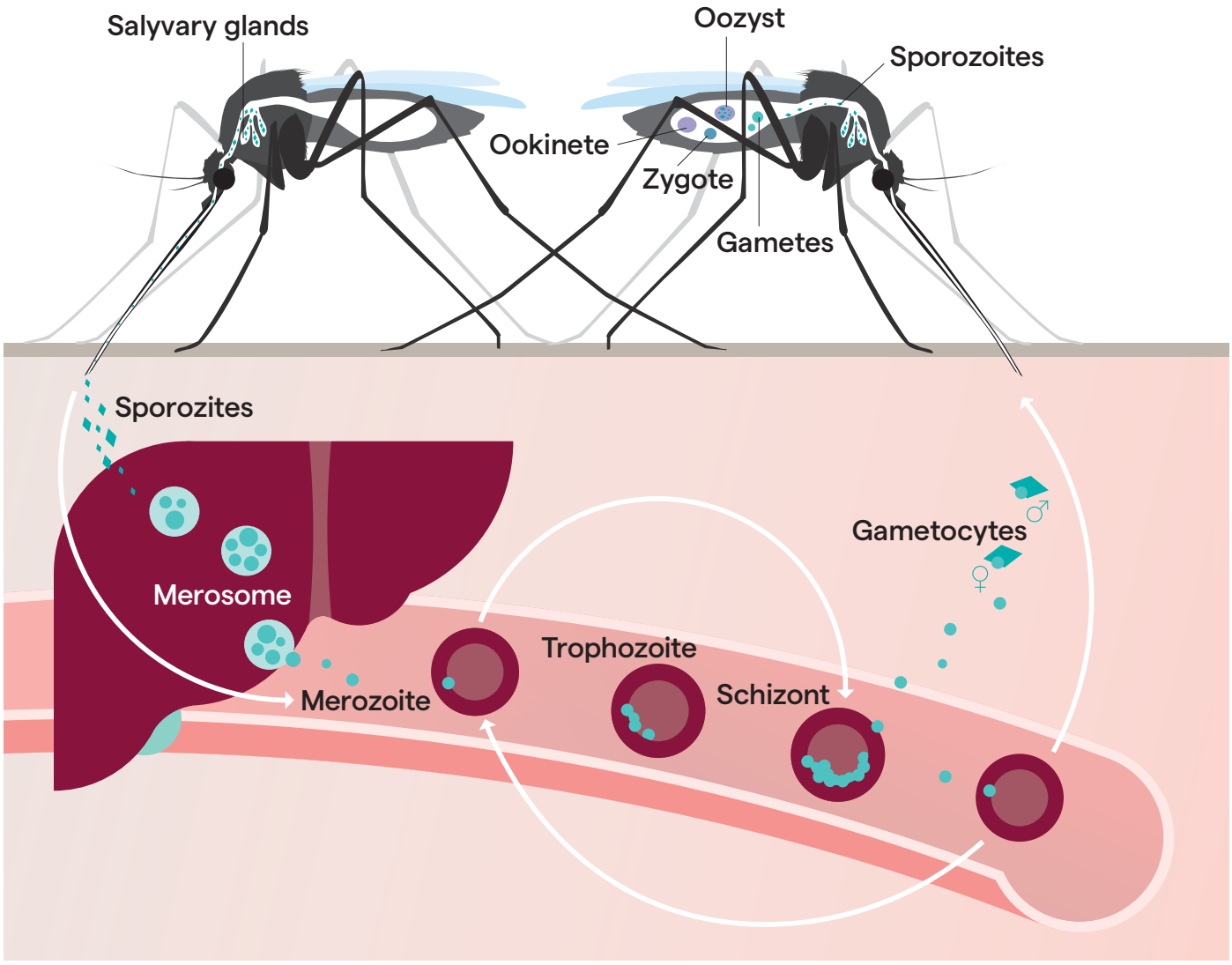


Figure 5.
A human is infected with malaria when an infected female Anopheles mosquito bites and injects Plasmodium sporozoites into the bloodstream. The sporozoites quickly make their way to the liver, where they infect the hepatocytes. Over the next week or so, the sporozoites multiply asexually in the hepatocytes. No symptoms are apparent at this stage. After they are released from the liver, the merozoites invade the bloodstream where they infect the erythrocytes. Some merozoites replicate asexually in the erythrocytes and are released back into the bloodstream when the erythrocyte ruptures, causing the classical symptoms of malaria. However in other erythrocytes, instead of replicating asexually, the merozoites develop into gametocytes, the sexual form of Plasmodium. The gametocytes are picked up by a mosquito that bites an infected person and undergo a sexual reproduction cycle in the mosquito, producing more sporozoites. The cycle begins again when another human is bitten.

Table 6. Disease models

Applications	Description	Hepatocyte Formats
Non-alcoholic steatohep-atitis (NASH) models	Steatosis can be induced in hepatocytes using a variety of small molecule or nutrient inducers.	Highest quality plated monolayer cultures 3D cultures/Spheroids Co-Cultures
Diabetes models	Hepatocytes respond to insulin by altering their glucose and fat metabolism. Various assays to induce lipogenesis and gluconeogenesis in hepatocytes are used.	Highest quality plated monolayer cultures 3D cultures/Spheroids Co-Cultures

The challenge of using hepatocytes for research on Plasmodium infection has been that the hepatocytes must survive for several weeks in culture. Recent work has shown that co-cultures of cryopreserved hepatocytes with liver Kupffer cells (i.e., the resident macrophage in the liver) can successfully mimic the liver stage of the Plasmodium life cycle[18].

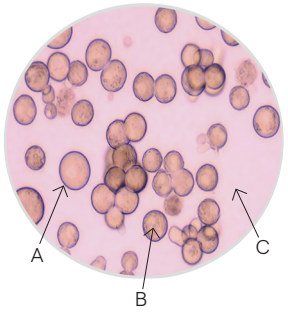
Non-alcoholic Steatohepatitis (NASH) Models

Non-alcoholic steatohepatitis (NASH) is a disease of the liver characterized by the infiltration of inflammatory cells, increased lipidosis in the hepatocytes, and early stages of collagen deposition leading to fibrosis. NASH is a progression of non-alcoholic fatty liver disease (NAFLD), which is associated with obesity and diabetes. The incidence of NAFLD is thought to be as high as 30% of the population and continues to rise with the co-incident rise in obesity

The interest in developing cell-culture models for NASH is very high. Currently, a non-invasive diagnostic is not available for NASH and effective treatments are still in the early research and development stages. However, the onset of fibrosis, which is characterized by increased collagen deposition, is a hallmark pathological feature of NASH. Cell models that recapitulate the features of NASH contain both hepatocytes and stellate cells in co-culture or in 3D models[19].

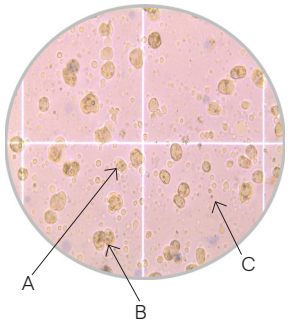
What Makes a Good Hepatocyte Preparation?

Happy



- Well-defined membranes
- Rounded shape (A)
- Minimal cell debris or excretion products (C)
- Clear cytoplasm (B)
- >75% viability using standard WEM or DMEM media
- No more than a 20% drop in viability over 2 hours

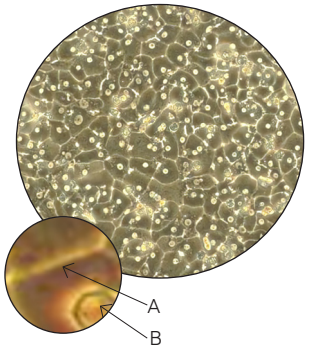
Not happy



- Cell membrane integrity is compromised (A)
- Cytoplasm inclusions (swollen organelles) (B)
- Cell debris (C)
- Low cell viability
- Cell shape compromised (A)

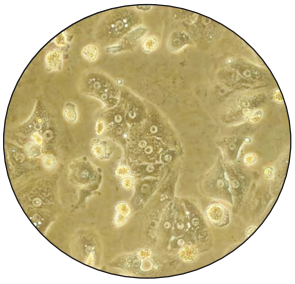
What is a Good Hepatocyte Culture?

Ideal



- Edge-to-edge monolayer (100%)
- Nuclei are bright and distinct (B)
- Tight cell-cell contact
- "Cobblestone" appearance
- Bile duct formation (A)

Not ideal



- Poor monolayer confluency (20%)
- Cells are elongated ("fibroblast like") (A)
- Large gaps between cells (B)

Figure 6.
The cellular morphology of hepatocytes is a good indicator of hepatocyte health in both suspension preparation and in plated monolayer or 3D culture.

How to Select the Right Batch of Hepatocytes for Your Needs

The first step in lot selection should be to determine the duration of the assay and whether a plateable qualified lot is essential. Typically, if the assay is for metabolic or transporter activity and can be done in a short time window of less than 4 hours, a suspension qualified lot will suffice. For most other applications, identifying a plateable qualified lot is essential.

For all hepatocytes, a key component to selecting a lot is to visualize a microscopic examination of the cellular morphology. Figure 6 is a comparison of the cellular morphologies in good and poor hepatocyte preparations, and cells in ideal and less-than-ideal hepatocyte cultures. Figure 7 is a flow chart for the selection of a lot of human primary hepatocytes.

Primary human hepatocytes can show significant lot-to-lot variability due to the nature of the isolation procedures and hepatocyte function. The lot-to-lot variability can be attributed to donor-to-donor variability, inconsistency in the health of the tissue, and treatments that the donor may have undergone near the time of donation. As a result, hepatocyte lots must be characterized for many different functions in order to provide users with important supplemental data about the health of the cells in addition to the donor demographics.

Each hepatocyte lot should be characterized for post-thaw viability and viable cell yield. Additionally, the cells must be characterized for their ability to form a monolayer in culture. Primary hepatocytes maintain their function for a much longer time when they are able to form tight junctions with other hepatocytes. Therefore, the plating efficiency should be an important factor in selecting an appropriate lot of hepatocytes. As previously noted, primary hepatocytes have many applications, but drug metabolism studies tend to be the most common.

Hepatocytes to be used for basal metabolic activity measurements and metabolite identification should also be evaluated for metabolic competency in terms of the common CYP enzymes, such as CYP1A2, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP3A, which are typically involved in the metabolism of pharmaceuticals.

The regulatory agencies have issued official guidance about the performance of drug interaction studies using hepatocytes *in vitro*. A hepatocyte lot should demonstrate a relevant level of induction of CYP1A2, CYP2B6, CYP2C8, and CYP3A4 in response to positive control inducers. (9)

As spheroids become more widely utilized as models in drug metabolism, toxicity, and disease models, verification that the batch of hepatocytes will readily form spheroids will be an important parameter. To date, its been observed that only nicely plateable batches of hepatocytes are capable of forming spheroids on a consistent basis. Even then, the formation and functionality can still show significant variability.

Ultimately, the recommended approach for the selection of the best lot is to test several different batches in a pilot assay to determine which is most suitable for your specific application.

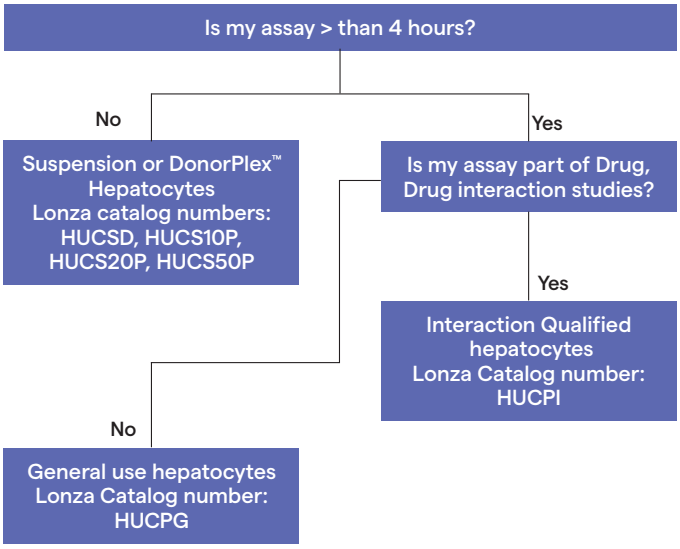


Figure 7.
Lonza provides different human primary hepatocytes for different applications. Use this flowchart as a guide to find the appropriate cells for your application, or call Lonza Scientific Support.

Successfully Culturing Primary Hepatocytes

To improve successful culturing of hepatocytes, below is a troubleshooting guide and frequently asked questions to supplement information provided in the downloadable protocols.

The health of the hepatocytes is dependent upon following Lonza's provided protocols carefully. The most up-to-date and downloadable versions of these protocols can be found here:

bioscience.lonza.com

Frequently Asked Questions

1. **Why is it important to use collagen-coated tissue culture plates?**
   Hepatocytes *in vivo* are connected together in a collagen rich environment. Unlike tumor cells and many other cell-types, tissue-culture treated plastic is not sufficient to support the attachment of primary hepatocytes. Corning® BioCoat™ plates coated with rat-tail Collagen Type I are recommended.

2. **How long are cryopreserved hepatocytes viable in liquid nitrogen?**
   When stored at the proper storage conditions (< -150° C), the industry standard is 10 years. The duration of viability in liquid nitrogen may have lot-to-lot variability and is not formally tested.

3. **What is "sandwich culture?"**
   Sandwich culture refers to the laying of hepatocytes on top of a collagen matrix and then layering another basement membrane extract (BME) on top of the cells. Committing the cells to be surrounded on all sides by extracellular matrix encourages membrane polarization, tight junctions between adjacent cells, and the formation of bile pockets and sometimes bile canals. Only when in this configuration are membrane transporters appropriately expressed and functional.

4. **Can I repeatedly thaw and refreeze cryopreserved hepatocytes if I only need to remove a small aliquot of these products from a vial?**
   No. Repeated freezing and thawing causes extremely low recovery of viable hepatocytes. That is why we pack our cryopreserved hepatocytes in a volume that is convenient for most single-use applications.

5. **What information is available about the human donors from which the hepatocytes were prepared?**
   The following donor demographics are typically made available to researchers:

   - Age
   - Gender
   - Race
   - BMI
   - Tobacco Use
   - Alcohol Use
   - Drug Use
   - Serologies – including HIV, Hepatitis B, Hepatitis C, EBV (Epstein- Barr), and CMV (Cytomegalovirus). For ethical and privacy reasons we do not provide any information which might identify the donor.

6. **Can I store cryopreserved hepatocytes in the cryoshipper?**
   No, the cryoshipper is not intended for storing cryopreserved hepatocytes. Upon receiving your shipment, the cryopreserved hepatocyte vials must be removed and stored at temperatures lower than -150°C. The cryoshipper should then be returned following instructions included in the shipment.

7. **Where does Lonza get its human tissues?**
   Lonza has established a network of sources for non-transplantable organs. All whole liver tissues that we receive are not transplantable and the donor or next of kin has consented to donating their tissue for research applications. Lonza only receives liver tissue from approved and US government regulated sources under the strictest ethical guidelines.

For more answers to Frequently Asked Questions regarding primary hepatocytes and other products, please visit our FAQ Database:

www.lonza.com/faq

Guide for Troubleshooting Problems Encountered When Culturing Hepatocytes

Problem	Possible Reasons	Possible Solutions
Low Viability after thawing, before plating	The hepatocytes remained in the warmed DMSO-containing cryopreservation medium too long	Carefully thaw in a clean water bath set no higher than 37° C
		Watch for ice crystals to thin and remove from water bath when small spindle of ice remains
		Work quickly to transfer cells into thawing medium after thawing in a water bath
	Trypan blue concentration is too high	Ensure that final trypan blue concentration in counting medium is no more than 0.04%
	Cells were vortexed to resuspend	Following centrifugation, we recommend flicking or 'ring the bell' for homogenization in the cell counting tube. For post-centrifugation, a gentle rocking of the conical tube is recommended
	Cells were not stored properly	Store cells in liquid nitrogen vapor at all times even during transfer steps.
	Cells were transferred using narrow bore pipet	Pour cells slowly thawing medium. Or when using a pipet, very slowly pipet the contents and use a wide-bore tip whenever possible
Plateable cells have poor attachment/No cells attached to plates	Tissue culture plates are not collagen coated	Use collagen coated plates such as Corning®, BioCoat™
	Cell density is not accurate	Check calculations to ensure that cell density matches recommended values
	Plating medium did not contain appropriate ingredients to promote attachment	Use MP100 or MP250 plating mediums
Plateable cells unevenly plated in cell culture wells	Cells were not evenly distributed during plating	Use a shaking method where plate is setting on a flat surface and pushed back and forth in a north-south, then east-west
Cells detach after first day	Environmental conditions not optimal	Calibrate CO_2 levels and humidity in incubator
	Maintenance medium did not contain appropriate ingredients to promote healthy cultures	Use HCM™ Bulletkit® and ensuFre all SingleQuots® ingredients are added
Cells do not maintain metabolic activity long enough	Cells do not form correct cell-cell interactions that help to maintain hepatocyte phenotype	Use Matrigel® Overlay to promote tight junctions between cells
		Check cell density calculations to ensure a confluent monolayer is formed immediately after attachment
Cell monolayer is coated with darkly colored debris and dead cells	Dead cells remain attached to the live cells during plating	Perform north-south, then east-west shaking at 15 minute intervals during the first hour after plating to allow live cells to attach and dead cells to release and make room. Change media to fresh plating medium after live cells attach in the first hour, then to maintenance medium after 4 – 6 hours
	Cells were overseeded	Check calculations to ensure that cell density matches recommended values

References:

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Hepatocyte Products

Cat. No.	Product Name	Description
Human Cryopreserved Hepatocytes		
HUCPI	Human Hepatocytes, Interaction Qualified	Cryopreserved, plateable
HUCPG	Human Hepatocyte, General Purpose	Cryopreserved, plateable
HUCSD	Human Hepatocytes	Cryopreserved, suspension
HUCS10P	DonorPlex™ Hepatocytes, 10 donor pool	Cryopreserved, suspension
HUCS20P	DonorPlex™ Hepatocytes, 20 donor pool	Cryopreserved, suspension
HUCS50P	DonorPlex™ Hepatocytes, 50 donor pool	Cryopreserved, suspension
Human Cryopreserved Non-Parenchymals		
HUCNP	Human Liver Non-Parenchymal Cells, 2e5 per vial	Cryopreserved
HLKC-200K	Human Kupffer Cells , 2e5 per vial	Cryopreserved
HLKC-500K	Human Kupffer Cells, 5e5 per vial	Cryopreserved
HLECP1	Human Liver-Derived Endothelial Cells	Cryopreserved
HUCLS-200K	Human Stellate Cells, 2e5 per vial	Cryopreserved
HUCLS-1M	Human Stellate Cells, 1e6 per vial	Cryopreserved
NoSpin HepaRG™ Cells and Medium		
NSHPRG	NoSpin HepaRG® Cells	Cryopreserved
MHTAP	HepaRG® Thaw and Plating Medium Supplement	Base medium additive for thawing and plating
MH100	HepaRG® Base Medium with Supplement	Serum-free base medium
MHIND	HepaRG® Induction Medium Supplement	Base medium additive for induction assays
MHMET	HepaRG® Maintenance/Metabolism Supplement	HepaRG® Maintenance/metabolism supplement
MHPIT	HepaRG® Pre-Induction and Tox Medium Supplement	Base medium additive for toxicity assays
Hepatocyte Medium		
CC-3198	HCM™ Hepatocyte Culture Medium BulletKit® Kit	Includes basal medium and SingleQuots® Kit
MM250	Hepatocyte Maintenance Media	For all hepatocytes
MP100	Hepatocyte Plating Medium	For all hepatocytes
MCHT50	Human Hepatocyte Thawing Medium	For cells HUCPI, HUCSD, HUCPG , HUCS50P
MCHT50P	Pooled Human Hepatocyte Thawing Medium	For cells HUCS10P, HUCS20P
MCST250	Human Stellate Cell Growth Media	For cells HUCLS

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