PROVEN GLOBAL CONTRACT RESEARCH EXPERTISE
FROM DISCOVERY THROUGH CLINICAL SUPPORT

Microsomal Protein Binding of Drugs

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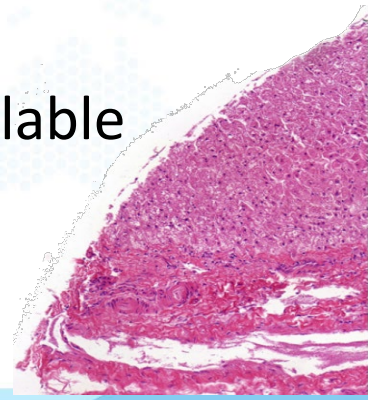
Overview

- What is microsomal protein binding (MPB)?
- Why is measuring MPB important?
- Methods to measure MPB
- MPB study design
- Data example

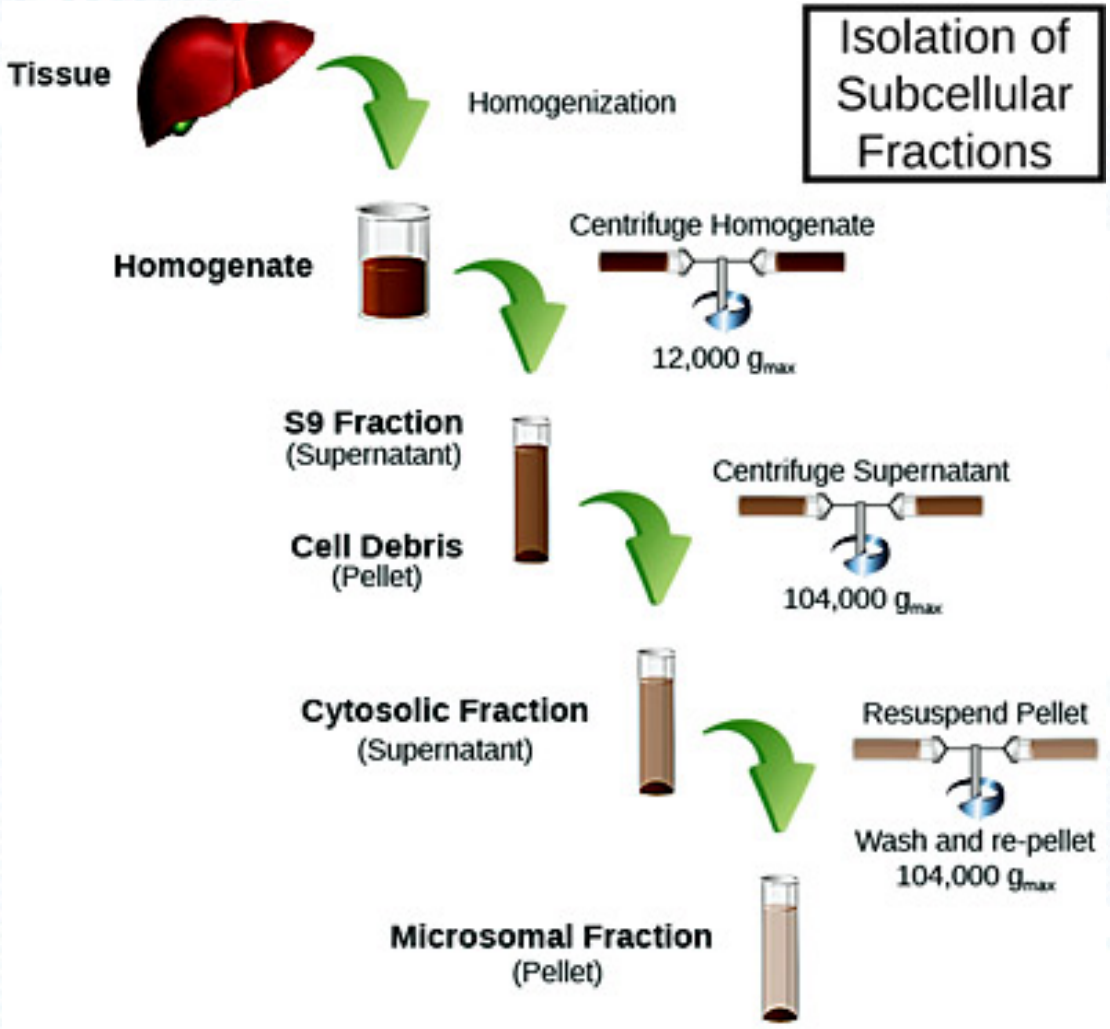
What are microsomes?

- Microsome is a type of hepatic in vitro test system used to evaluate the drug interaction potential of an investigational drug.
- Microsomes are the test system in drug inhibition and drug metabolism studies.
- Formed from the endoplasmic reticulum when cells are disrupted and the homogenate is subjected to differential centrifugation.
- Contain CYP and UGT enzymes amongst others.
- Drug that is sequestered in microsomes *in vitro* is unavailable for direct interaction with metabolizing enzymes.



Preparation of microsomes from hepatocytes

Differential centrifugation



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Why measure microsomal protein binding?

1. **2020 FDA Guidance**

> Microsomal protein concentrations are usually less than 1 mg/mL. The sponsor should correct for nonspecific binding during the incubation if this binding is expected to influence the analysis of kinetic data. Nonspecific binding can be measured experimentally (e.g., using equilibrium dialysis or ultrafiltration) (Hallifax and Houston 2006) or predicted using in silico methods. It is recommended to experimentally determine nonspecific binding for highly lipophilic drugs (Gertz, Kilford, et al. 2008).

As per the FDA Guidance, measurement of MPB is required for Drug Inhibition studies, but not for Drug Metabolism studies

2. Better understanding of relationship between in vitro metabolism and in vivo pharmacokinetics

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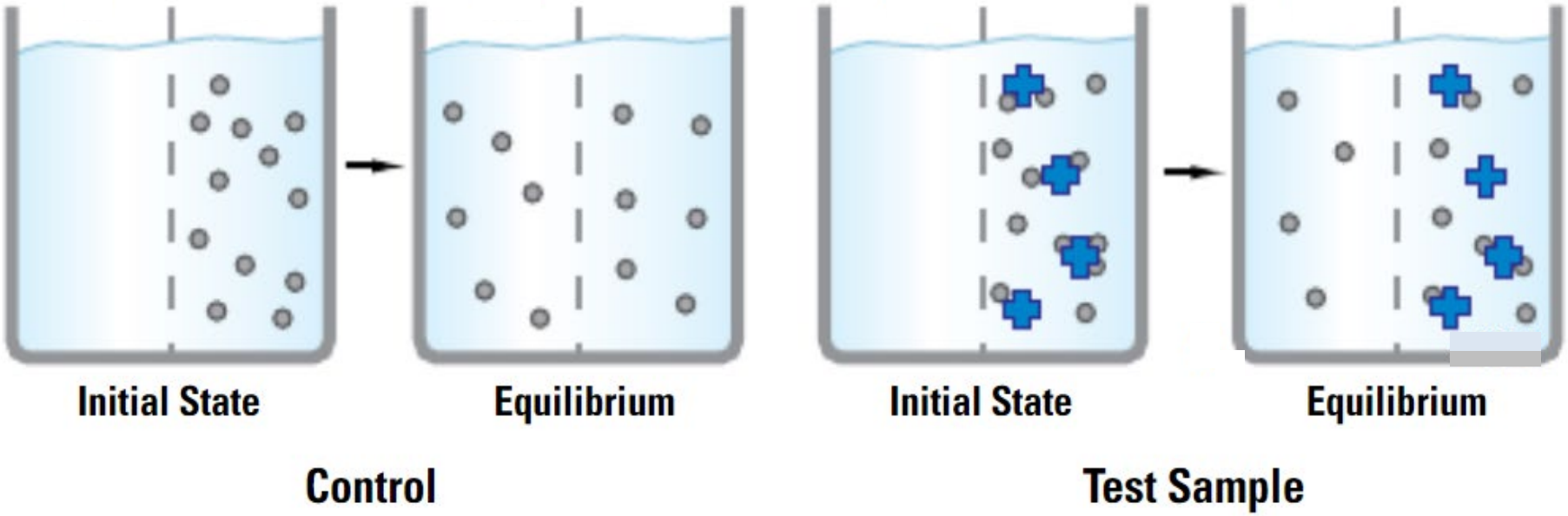
Methods to determine MPB

- Equilibrium Dialysis
- Ultracentrifugation
- Ultrafiltration

Principle:

Physical separation and measurement of the unbound and bound drug to calculate their relative fractions.

Equilibrium Dialysis



- Drug
- Microsomal protein

Rapid Equilibrium Dialysis (RED) Device

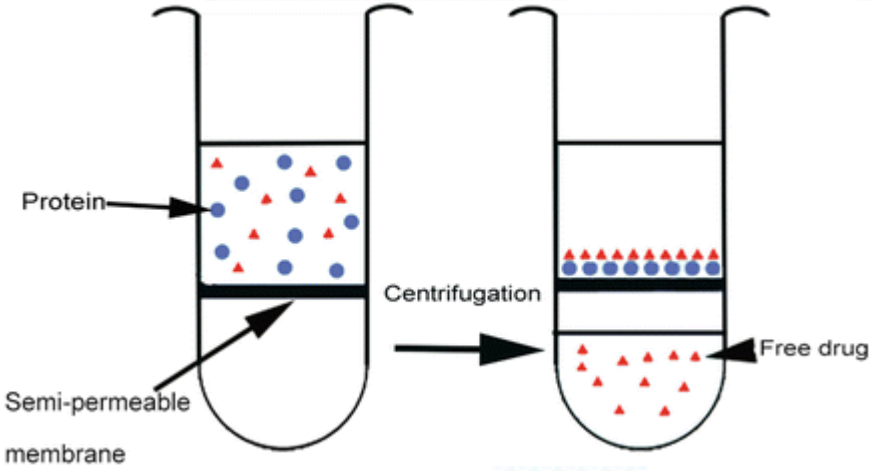
- Base plate has a 96-well footprint
- Holds 48 RED devices
- Dialysis membrane (MWCO 8K, 12K)
- Multiple sampling

Drawbacks:

- Non specific binding to membrane- underestimate fraction unbound
- Protein leakage- overestimate fraction unbound
- Volume shifts - dilute protein concentration.

Ultrafiltration

Separation of the protein and bound drug from free drug in solution occurs using a suitable membrane which retains the proteins and is assisted by positive pressure or centrifugation (2000 g).



Advantage:
Fast process

Drawbacks:
- Non specific binding to membrane- underestimate fraction unbound
- Molecular sieving (water from plasma passes through the membrane faster)- underestimate Fu

Ultracentrifugation

Spike test article in buffer containing microsomes

Centrifuge ~ 500,000 g for 5-6 hours at 37° C

Aliquot of supernatant

Determine levels by LC/MS

Advantage:

No non-specific binding

Disadvantages:

- Long process
- Cosedimentation of free and bound drug

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Study Design

Microsomal protein binding studies are offered as a stand-alone study or may be part of a drug inhibition study.

Selection of drug concentrations:

- Stand-alone: Client request
- Within drug inhibition protocol: Microsomal protein binding will be assessed at the IC_{50} concentration with same human liver microsomal protein concentration as the IC_{50} assay.

Impact of the microsomal binding will be discussed in the report.

Study Design

Human liver microsomes
Incubation time: 4h, 6h, 24h
1 Concentration

CYP/ UGT buffer
Incubation time: 4h, 6h, 24h
1 Concentration

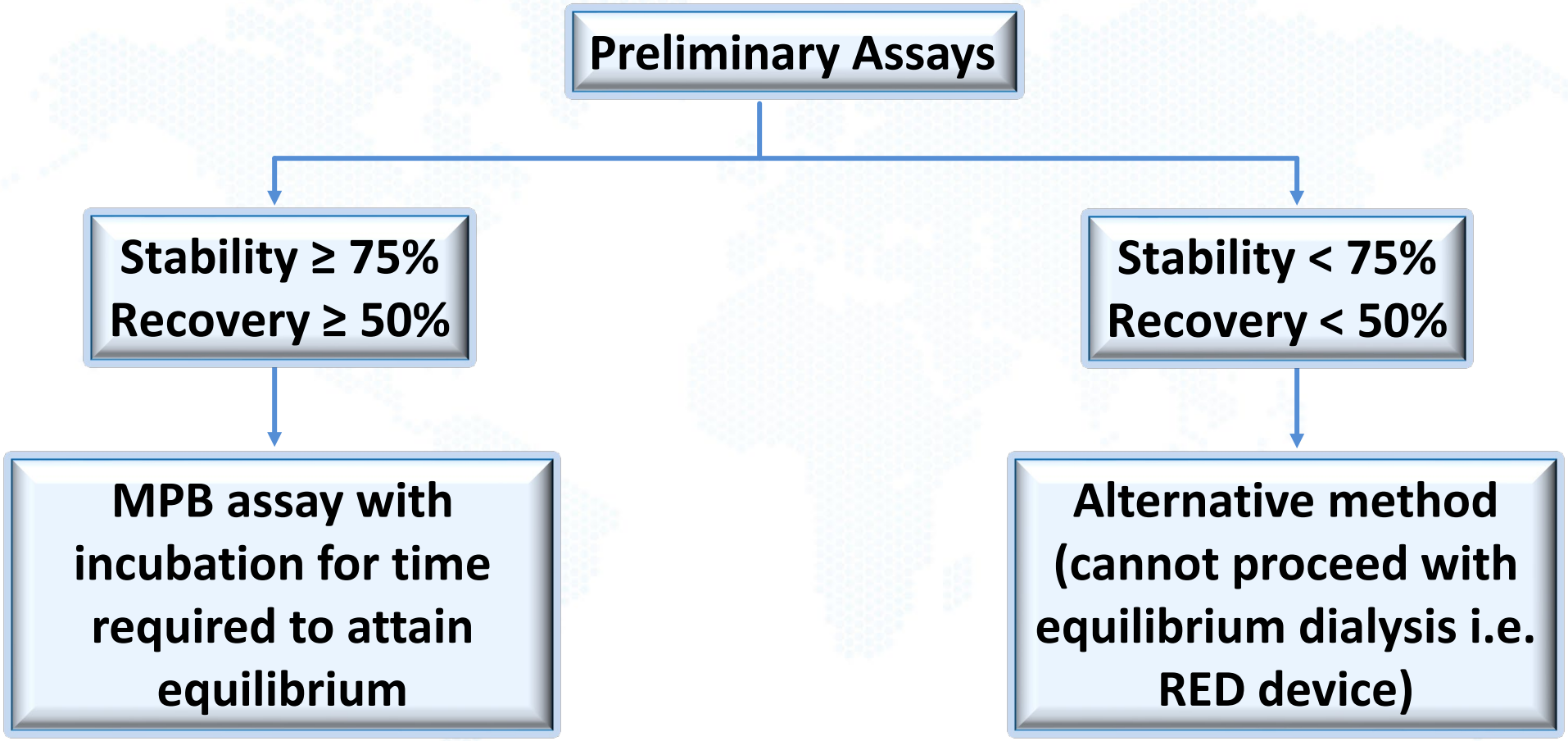
Preliminary Assays

Stability (optional)
Non-specific binding
Time to reach equilibrium

Microsomal protein binding Assay

Fraction unbound

Decision tree



Data example

1 µM paroxetine with	Buffer	Run	Fu	Mean Fu ± RSD (inter-day)	Binding (%)	Mean % bound ± RSD
0.1 mg/mL Human liver microsomes	CYP	1	0.476	0.456 ± 3.9	52.4	54.4 ± 3.3
		2	0.449		55.1	
		3	0.442		55.8	
1 mg/mL Human liver microsomes		1	0.066	0.0657 ± 5.85	93.4	93.4 ± 0.4
		2	0.069		93.1	
		3	0.062		93.8	
0.1 mg/mL Human liver microsomes	UGT	1	0.623	0.631 ± 4.5	37.7	36.9 ± 7.7
		2	0.607		39.3	
		3	0.662		33.8	
1 mg/mL Human liver microsomes		1	0.115	0.118 ± 2.2	88.5	88.2 ± 0.3
		2	0.119		88.1	
		3	0.120		88.0	

Summary of variables in microsomal protein binding studies

Protein: Human liver microsomes
Microsomal concentrations: 0.1 or 1 mg/mL
Buffer: CYP or UGT
Positive control: 1 µM paroxetine

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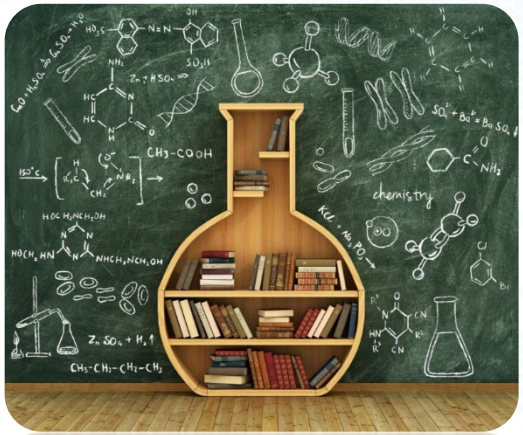
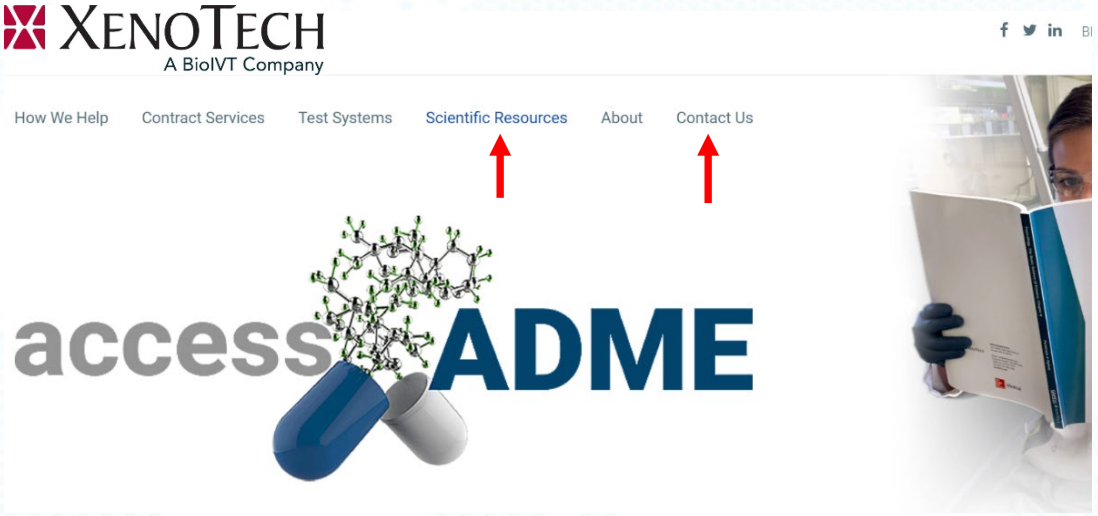
For questions or further resources





Thank You!