

# I-SCREEN: AN EX VIVO HUMAN MICROBIOME PLATFORM TO STUDY MICROBIOME INDUCED REVERSE METABOLISM OF METABOLITES BACK TO PARENT

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## INTRODUCTION

- Gut microbiota plays a major role in metabolizing xenobiotics into active, inactive or toxic metabolites, thereby influencing pharmacokinetics, efficacy and toxicity profiles of prescribed drugs, which is currently being underexplored.
- We have previously shown metabolism by selected drugs by pooled microbiota in the i-screen platform (van de Steeg et al., 2018).



## AIM

- To study the fate of drug metabolites (generated in the liver) upon exposure to gut microbiota in the i-screen platform, a translational intestinal multi-well screening platform simulating the human colonic microbiota conditions.

## METHODS

- The metabolic capacity of gut microbiota was investigated by incubating 8 drug metabolites (5 glucuronides, 2 N-oxides and 1 sulphate, Table 1) in i-screen using pooled human colonic microbiota (pool of 7 healthy adult fecal samples) for 24 hours under fully anaerobic conditions [1,2] (Figure 1), followed by LC-HRMS analysis of the samples. Samples were taken at 0, 6 and 24 hours. This allowed for investigation of different microbial transformations that may occur in the human gut

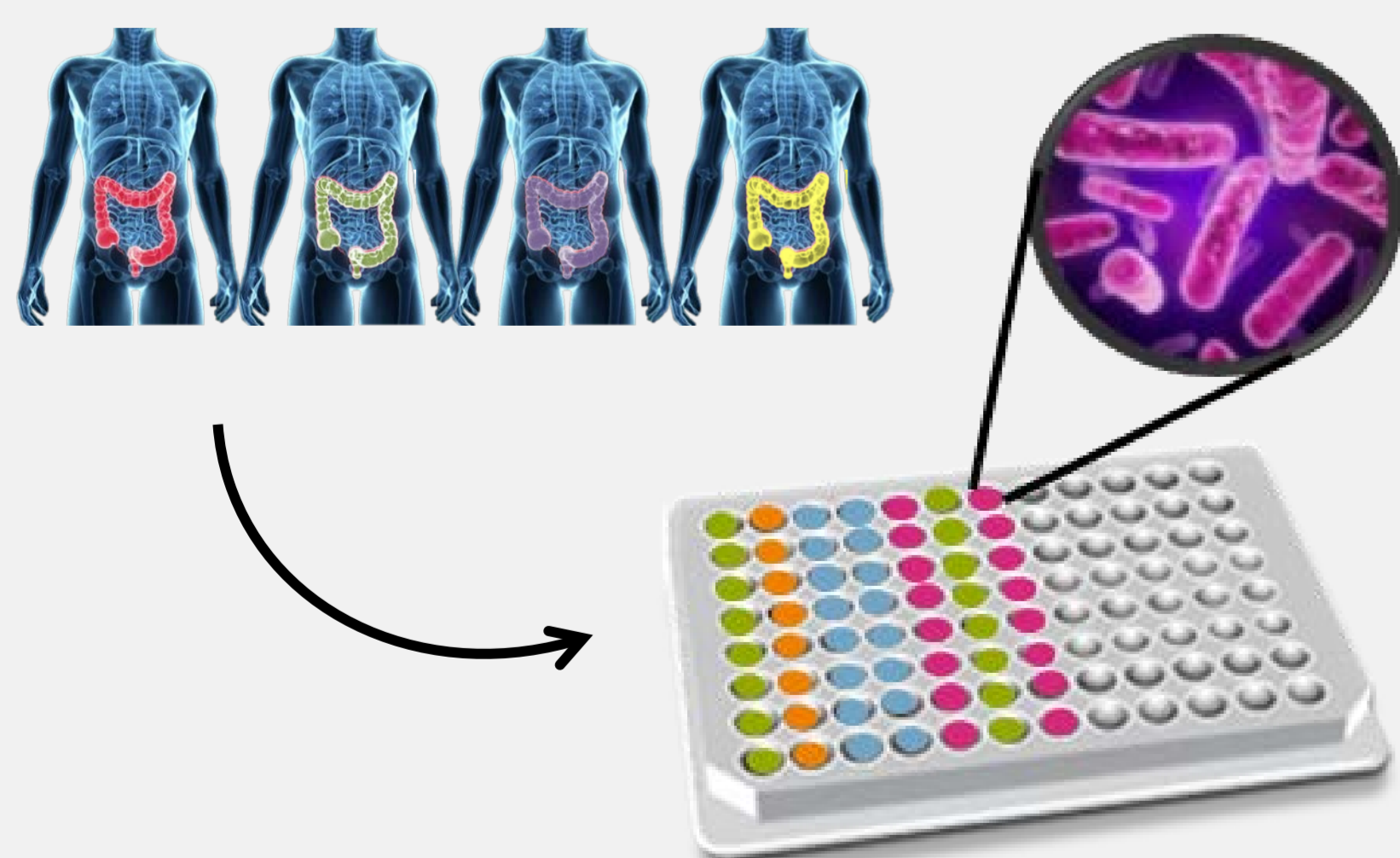


Figure 1. Schematic representation of (personalized) i-screen 96-well Format

## REFERENCES

- <sup>1</sup> Ladirat SE et al. *J. Micr. Methods* (2013) 92: 387-397  
<sup>2</sup> Van de Steeg E et al. *Drug Metab Dispos* (2018) 46: 1596-1607

## RESULTS

- Previously we have shown that out of 12 parent drugs 5 were metabolized in i-screen experiments and specific reduction metabolites could be shown (microbiota-induced metabolism was demonstrated for risperidone, sulindac, sulfapyrazone, nizatidine and sulfasalazine).<sup>2</sup>
- Here we have analyzed the capability of adult human colon microbiota to metabolize drug metabolites generated in the liver back to their respective parent drugs, since these drug metabolites may through biliary excretion end up in the GI-tract in vivo.

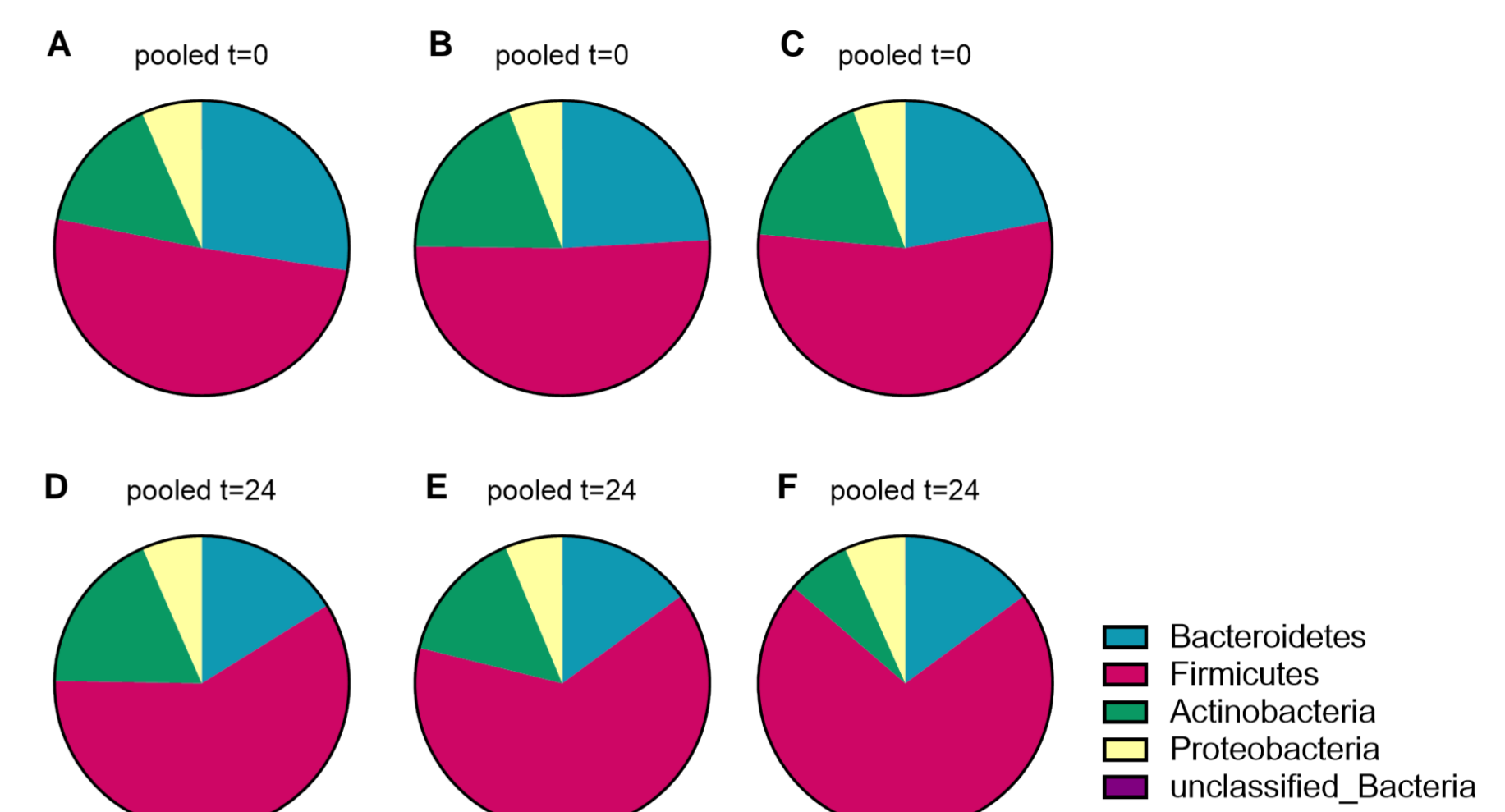


Figure 2. Microbial composition of pooled human colon microbiota before (t=0) and after ex vivo fermentation in i-screen for 24 hours (t=24). Intra-experimental variation is shown by presenting A, B and C and D, E and F representing triplicate incubations of one experiment. Data are presented as mean relative abundance of the individual microbial species (n=3).

Table 1. Overview of metabolic fate of 8 Investigated Drug metabolites Susceptible to Microbial Metabolism in i-screen

Substrate (metabolite)	Product (parent drug)	Reaction Type	Observation
Acetaminophen sulfate	Acetaminophen	Phenol sulfate hydrolysis	<ul style="list-style-type: none"> <li>• Reaction was completed at 6hr</li> <li>• No reaction in microbiota free incubation</li> </ul>
Mycophenolic Acid Glucuronide	Mycophenolic Acid	Acyl glucuronide hydrolysis	<ul style="list-style-type: none"> <li>• Reaction occurs at 6hr and continued over 24 hr period</li> <li>• No reaction in microbiota free incubation</li> </ul>
Sertraline-N-Carbamoyl Glucuronide	Sertraline	Carbamoyl glucuronide hydrolysis	<ul style="list-style-type: none"> <li>• Very fast reaction, small amount of product observed in t=0, but not in microbiota free incubation</li> <li>• Reaction was completed at 6hr</li> <li>• Sertraline was further consumed between 6 and 24 hr</li> </ul>
Benzydamine N-Oxide	Benzydamine	N-Oxide Reduction	<ul style="list-style-type: none"> <li>• Reaction was completed at 6hr</li> <li>• some reaction occurred in microbiota free incubation (instability)</li> <li>• Benzydamine was further consumed between 6 and 24 hr</li> </ul>
Imipramine N-Oxide	Imipramine	N-Oxide Reduction	<ul style="list-style-type: none"> <li>• Reaction was completed at 6hr</li> <li>• some reaction occurred in microbiota free incubation (instability)</li> <li>• Imipramine was further consumed between 6 and 24 hrs</li> </ul>
SN-38 Glucuronide	SN-38	Phenol Glucuronide Hydrolysis	<ul style="list-style-type: none"> <li>• Reaction occurs at 6hr and continued over 24 hr period</li> <li>• No reaction in microbiota free incubation</li> </ul>
Raloxifene 4'-Glucuronide	Raloxifene	Phenol Glucuronide Hydrolysis	<ul style="list-style-type: none"> <li>• Reaction occurs at 6hr and continued over 24 hr period</li> <li>• No reaction in microbiota free incubation</li> </ul>
Raloxifene 6-Glucuronide	Raloxifene	Phenol Glucuronide Hydrolysis	<ul style="list-style-type: none"> <li>• Reaction occurs at 6hr and continued over 24 hr period</li> <li>• No reaction in microbiota free incubation</li> </ul>

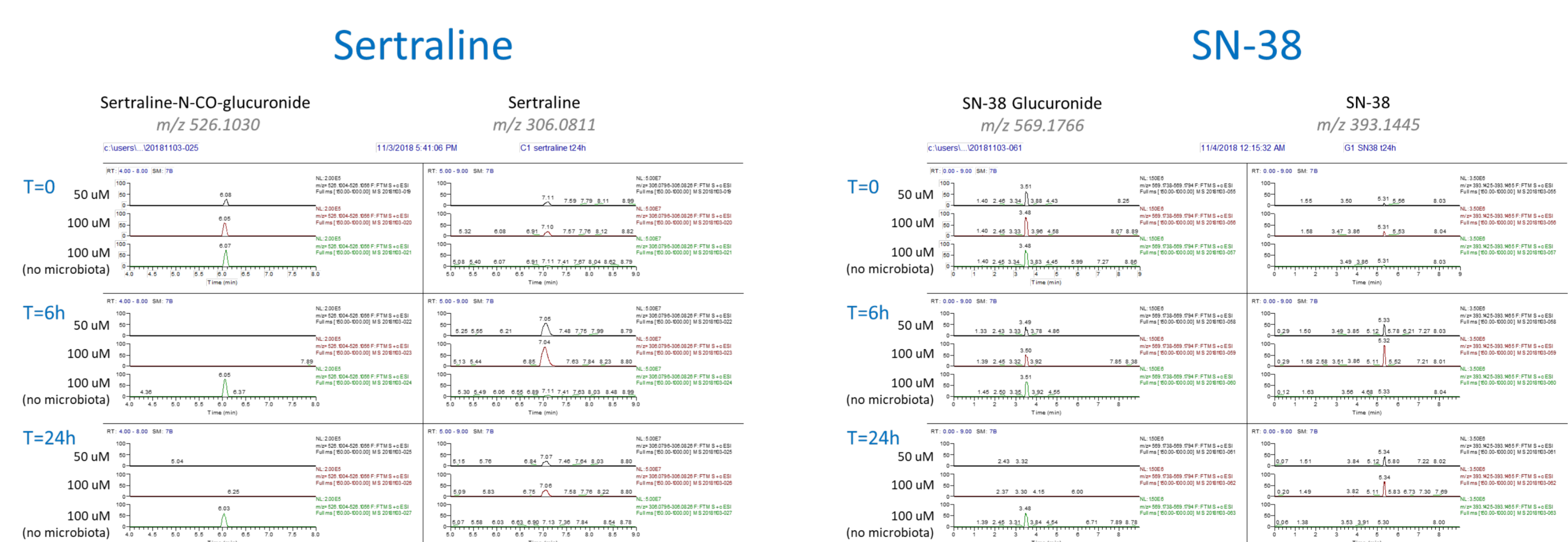


Figure 3. Metabolic fate of sertraline N-carbamoyl glucuronide and SN-38 glucuronide in i-screen

## CONCLUSIONS

- Colon microbiota can metabolize parent drugs which enter the colonic environment, but also back-metabolize certain metabolites to their parent drugs
- These metabolites are generally formed in the liver, secreted into the bile, then back-converted in the GI-tract and re-absorbed, a process referred to as enterohepatic circulation (EHC)
- The i-screen platform can serve as an *in vitro* system to study this part of the EHC, in a potentially complementary approach that would utilize human hepatocytes to study initial metabolism and biliary transport component processes

## FUTURE PERSPECTIVES

- We are further exploring the potential for quantitatively analyzing metabolic reactions driven by the colonic microbiota
- We are actively investigating inter-individual differences in colonic metabolism (personalized medicine)
- Modulation of the microbiome to influence these metabolic processes offers interesting new treatment options