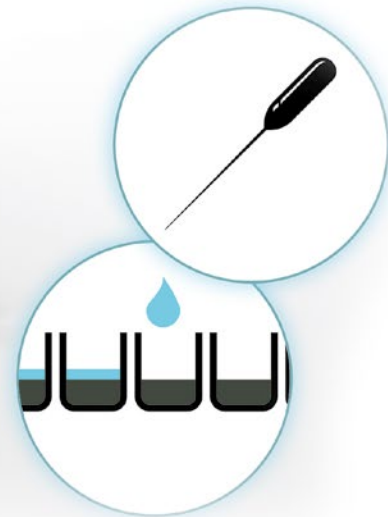


TNO I-SCREEN

INTESTINAL MICROBIOTA SCREENING PLATFORM FOR FUNCTIONAL INGREDIENTS



TNO innovation
for life

TNO's intestinal screening model (TNO i-screen) helps to quickly identify food ingredients that modulate the intestinal microbiota composition. For manufacturers, searching for health-promoting ingredients is a complex and time-consuming process. Large numbers of substances have to be screened, while for some components almost no proper identification methods are available. When a functional ingredient has finally been selected, extensive *in vitro* and human volunteer studies are required to demonstrate its safety and to validate its efficacy prior to marketing it as beneficial to health.

The intestinal microbiota has a symbiotic relationship with its human host and has a substantial contribution to metabolic, immunological and other protective functions in healthy individuals. For example, the intestinal microbiota are involved in preventing calcium oxalate kidney stone disease by degrading oxalate [1] and provide us with essential vitamins and hormones. Stress induced gastro-intestinal damage can even be counteracted with beneficial microbes that aid in maintaining the structure and function of the gastrointestinal tract [2].

THE TECHNOLOGY EXPLAINED

The TNO i-screen is essentially a series of 96 wells each with a volume of about 1 ml containing *ex-vivo* intestinal microbiota. Each i-screen can be simultaneously exposed to a large number of different conditions, allowing for cost effective screening of interesting lead ingredients.

Subsequently, the effects can be monitored in different ways: zooming in on the shifts in the microbial diversity, changes in the formation of metabolites (e.g. SCFAs), or genomic or transcriptomic changes in the population. The start microbiota in the TNO i-screen can be a standardized *ex-vivo* intestinal microbiota pool collected from infants, healthy adults, obese or lean adults. To mimic the heterogeneity of the population intestinal microbiota from individual donors can also be used in each well.

BENEFITS

TNO's i-screen platform allows you to replace the use of animals, reduce the number of human volunteer studies and make better choices during product development. In fact, TNO's i-screen studies can add up to a considerable cost saving in your development process. In general, the technology contributes to

shorten the time-to-market of new products by several months. Experiments in TNO's i-screen do not require ethics approval and allow to work with early stage (development) compounds (before safety evaluation), labelled compounds or pathogens in combination with a large variety in faecal material.

EXAMPLES OF I-SCREEN

Example 1: Prebiotic effect of functional ingredients

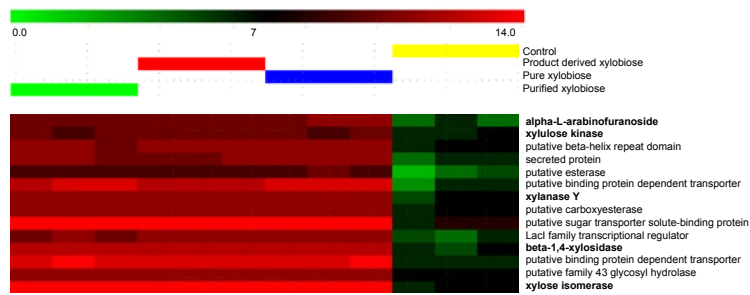
TNO has successfully applied i-screen in the investigation of prebiotic fibres such as fructo- and galacto- and xylose-oligosaccharides and many more health promoting ingredients such as green tea. Next to the observed bifidogenic effect, in the case of xylose oligosaccharide the metagenomic expression of xylose oligosaccharide degrading enzymes in Bifidobacteria was demonstrated, proving the relation between increase of Bifidobacteria and functional degradation of XOS. In a similar way i-screen can be used for the identification of novel ingredients for clinical and infant nutrition, or for the development of foods that increase the resistance of the elderly to disease-causing bacteria like *Clostridium difficile*.

Example 2: Restoring the intestinal microbiota after an antibiotic treatment

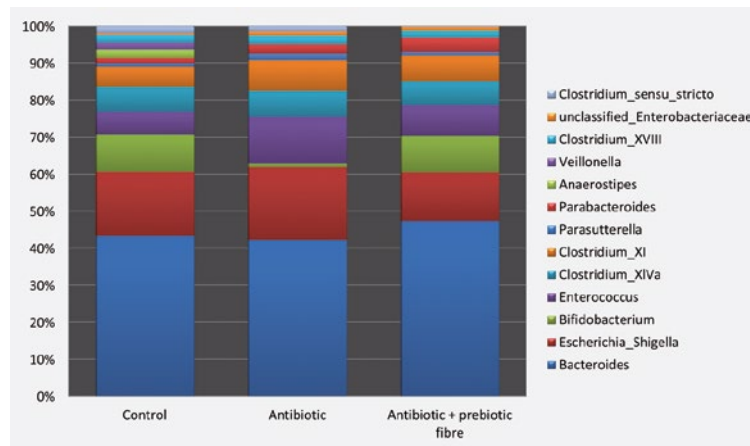
In the TNO i-screen it was demonstrated that the side-effects of the antibiotic amoxicillin was counteracted by the intake of certain prebiotics such as galacto-oligosaccharide (GOS). This was later confirmed in a double-blind, randomized parallel clinical study, in which the effects of the intake of GOS on the intestinal microbiota in twelve healthy adults receiving amoxicillin treatment was determined. These findings confirmed the TNO i-screen results, that GOS intake supports the recovery of the beneficial Bifidobacteria and indirectly the production of butyrate after amoxicillin treatment [3]. This enhances the potential effect of probiotic addition to an antibiotic-treated intestinal microbiota on the microbiota composition and activity.

TECHNICAL SPECIFICATIONS

- › 90 wells with 1 mL volume
- › Liquid and solid ingredients (only 10-50 mg required)
- › *In vitro* pre-digestion of ingredients (if required)
- › Possible Read-outs:
 - Microbial diversity (16S/ ITS2 amplicon sequencing)



Xylobiose induced gene expression in Bifidobacterium



The microbiome shift in Bifidobacterium is counterbalanced by a prebiotic fibre

- (Metagenomic) microbial expression profile using RNAseq
- Metabolic shifts by analysis of SCFA (acetate, propionate, butyrate), BCFA (iso-butyrate, iso-valerate), or numerous other metabolites
- › *Ex-vivo* intestinal microbiota pools collected from infants, healthy, obese or lean adults

COMBINED WITH OTHER TNO SERVICES

TNO's in house capabilities for microbiota analysis is a strong contributor to TNO i-screen's effectiveness. TNO i-screen can be combined with:

- › Advanced bioinformatics and statistics
- › Quantitative PCR for microbiota of interest

BUSINESS MODELS

This service is performed as contract research at TNO, however for some subjects open innovation collaborations are possible.

REFERENCES

1. Magwira et al. 2012. Appl. Microbiol. 113:418-428.
2. Lutgendorff et al. 2008. Curr. Mol. Med. 8:282-298.
3. Ladirat et al. 2014. Br. J. Nutr. 112:536-546.

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TNO HEALTHY LIVING

TNO initiates technological and societal innovation for healthy living and a dynamic society.

TNO
 Utrechtseweg 48
 3700 AJ Zeist
 The Netherlands
 P +31 (0) 88 866 1646

Edwin Abeln, PhD
 E edwin.abeln@tno.nl

North America
 Mark Posno, PhD
 E mark.posno@tno-na.com