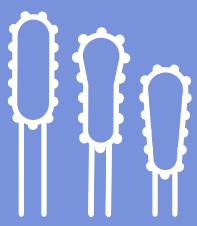
Scientific Booklet

Genetics & Microbiome



Copan

innovating together

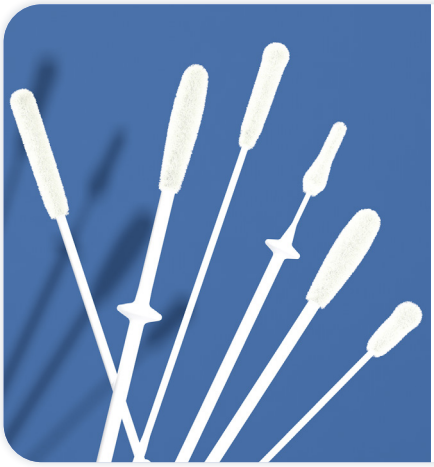
Acknowledgments:

We would like to express our gratitude to the authors whose works have been arranged in this booklet: their insights and expertise greatly assisted this prime selection. We are dedicated to developing and providing high-quality and cutting-edge biological sample collection products for human genomics, infectious diseases, environmental and forensic applications, along with automated workflow solutions. Copan's innovative approach enables an ever-expanding community of laboratories, scientists, and institutions to benefit from an accessible sample collection that guarantees reliable quality performance. Our goal is to continue this innovation by providing products, customized services, and prime solutions to improve patients' health and wellness. Over recent years, the studies within the microbiome field have multiplied, as microbiome connection to human health became evident. Although the capability to analyze the microbiome has expanded quickly, microbiome research remains a challenging task, and better methods of microbiome sampling, identification, and interpretation are then required. The same goes for genetic testing, which can diagnose genetic conditions or give information about your risk of developing cancer: genetic testing requires a clean, hDNA free sample collection to provide flawless results. We offer specific products to collect samples for microbiome research and genetic testing efficiently; in this booklet, you'll find a selection of the most interesting and recent independent studies where these products are used.

A patented technology

Why so special?

Copan conceived the FLOQ® technology in 2003, to answer the need of healthcare professionals for a more efficient sample collection. The main feature of this technology is the perpendicular arrangement of the short Nylon® fibers. This invention revolutionized the preanalytical world, bringing sampling to the next level.



FLOQSwabs®

FLOQSwabs® are made of a solid molded plastic applicator shaft with a tip, which they can both vary in size and shape. Thanks to their patented flocked tip, FLOQSwabs® ensure a flawless specimen collection, which expands downstream diagnostic testing capabilities.

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The combination of FLOQSwabs® with different Bacteriology and Virology transport media expands their flexibility even further.
Whether you have a specific collection site, pathogen, or downstream application in mind, Copan will be able to suggest the best combination to suit your needs.
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hDNAFree FLOQSwab®

Human hDNA Free FLOQSwabs® are a non-invasive, painless, and cost-effective system for DNA collection for genetic applications. Being sterile, free of amplifiable human DNA, free of detectable DNase and RNase, FLOQSwabs® hDNA free comprise essential requirements for various genetic applications, including microbiome research and genetic tests – such as food intolerance testing, genotyping, and HLA typing.

eNAT®

We specifically designed eNAT®, our nucleic acid collection and preservation system, to stabilize and preserve microbial and human nucleic acids for applications such as pathogen detection, predictive genetics, pharmacogenomics, HLA typing, and microbiome analysis. With its lysis and inactivation features, eNAT® is the ready-to-use device to quickly inactivation your sample, for a high-quality and unbiased nucleic acid yield and fast turnaround time from the sample to the response.

4N6FLOQSwabs®

4N6FLOQSwabs® Genetics is a complete line of FLOQSwabs® dedicated to DNA collection for human identification. 4N6FLOQSwabs® ensure that even minute amounts of DNA can be collected and remain available for testing. Moreover, a data matrix code ensures easy sample traceability, and our personnel STR database allows laboratories to verify whether an identified profile belongs to a Copan employee. Free of human amplifiable DNA, detectable DNase and RNase, 4N6FLOQSwabs® Genetics have been validated with numerous DNA extraction methods.

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PLoS One. 2018 Jul 23;13(7):e0201003

Genetics & Microbiome

Contraceptive Rings Promote Vaginal Lactobacilli in a High Bacterial Vaginosis Prevalence Population: a Randomised, Open-Label Longitudinal Study in Rwandan Women

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Keywords

FLOQSwabs®	Vaginal Lactobacilli	Contraceptive Rings	Rwandan Women

Abstract

Background: Hormonal contraception has been associated with a reduced risk of vaginal dysbiosis, which in turn has been associated with reduced prevalence of sexually transmitted infections (STIs), including HIV. Vaginal rings are used or developed as delivery systems for contraceptive hormones and antimicrobial drugs for STI and HIV prevention or treatment. We hypothesized that a contraceptive vaginal ring (CVR) containing oestrogen enhances a lactobacilli-dominated vaginal microbial community despite biomass accumulation on the CVR's surface.
Methods: We enrolled 120 women for 12 weeks in an open-label NuvaRing® study at Rinda Ubuzima, Kigali, Rwanda. Vaginal and ring microbiota were assessed collecting one cotton and two Copan FLOQSwabs® at baseline and each ring removal visit by Gram stain Nugent scoring (vaginal only), quantitative PCR for Lactobacillus species, Gardnerella vaginalis and Atopobium vaginae, and fluorescent in situ hybridization to visualize cell-adherent bacteria. Ring biomass was measured by crystal violet staining.
Results: Bacterial vaginosis (BV) prevalence was 48% at baseline. The mean Nugent score decreased significantly with ring use. The presence and mean log10 concentrations of Lactobacillus species in vaginal secretions increased significantly whereas those of G. vaginalis and presence of A. vaginae decreased significantly. Biomass accumulated on the CVRs with a species composition mirroring the vaginal microbiota. This ring biomass composition and optical density after crystal violet staining did not change significantly over time.
Conclusions: NuvaRing® promoted lactobacilli-dominated vaginal microbial communities in a population with high baseline BV prevalence despite the fact that biomass accumulated on the rings.

Sci Rep. 2019 Feb 18;9(1):2204

Genetics & Microbiome

The Endobiota Study: Comparison of Vaginal, Cervical and Gut Microbiota Between Women with Stage 3/4 Endometriosis and Healthy Controls

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Keywords

eNAT®	Endometriosis	Dysbiosis	Microbiota

Abstract

Dysbiosis in the genital tract or gut microbiome can be associated with endometriosis. We sampled vaginal, cervical and gut microbiota using Copan eNAT® kit from 14 women with histology proven stage 3/4 endometriosis and 14 healthy controls. The V3 and V4 regions of the 16S rRNA gene were amplifed following the 16S Metagenomic Sequencing Library Preparation. Despite overall similar vaginal, cervical and intestinal microbiota composition between stage 3/4 endometriosis group and controls, we observed diferences at genus level. The complete absence of Atopobium in the vaginal and cervical microbiota of the stage 3/4 endometriosis group was noteworthy. In the cervical microbiota, Gardnerella, Streptococcus, Escherichia, Shigella, and Ureoplasma, all of which contain potentially pathogenic species, were increased in stage 3/4 endometriosis. More women in the stage 3/4 endometriosis group had Shigella/ Escherichia dominant stool microbiome. Further studies can clarify whether the association is causal, and whether dysbiosis leads to endometriosis or endometriosis leads to dysbiosis.

Clin Infect Dis. 2020 Jun 10;70(12):2553-2560

Genetics & Microbiome

Each Additional Day of Antibiotics is Associated with Lower Gut Anaerobes in Neonatal Intensive Care Unit Patients

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Keywords

eNAT®	Gut Microbiome	Neonates	Intensive Care

Abstract

Background: Discontinuation of inappropriate antimicrobial therapy is an important target for stewardship intervention. The drug and duration-dependent effects of antibiotics on the developing neonatal gut microbiota needs to be precisely quantified.
Methods: In this retrospective, cross-sectional study, we performed 16S rRNA sequencing on stool swab (Copan eNAT®) samples collected from neonatal intensive care unit patients within 7 days of discontinuation of therapy who received ampicillin and tobramycin (AT), ampicillin and cefotaxime (AC), or ampicillin, tobramycin, and metronidazole (ATM). We compared taxonomic composition within term and preterm infant groups between treatment regimens. We calculated adjusted effect estimates for antibiotic type and duration of therapy on the richness of obligate anaerobes and known butyrate-producers in all infants.
Results: A total of 72 infants were included in the study. Term infants received AT (20/28; 71%) or AC (8/28; 29%) with median durations of 3 and 3.5 days, respectively. Preterm infants received AT (32/44; 73%) or ATM (12/44; 27%) with median durations of 4 and 7 days, respectively. Compositional analyses of 67 stool swab samples demonstrated low diversity and dominance by potential pathogens. Within 1 week of discontinuation of therapy, each additional day of antibiotics was associated with lower richness of obligate anaerobes (adjusted risk ratio [aRR], 0.84; 95% confidence interval [CI], .73–.95) and butyrate-producers (aRR, 0.82; 95% CI, .67–.97).
Conclusions: Each additional day of antibiotics was associated with lower richness of anaerobes and butyrate-producers within 1 week after therapy. A longitudinally sampled cohort with preexposure sampling is needed to validate our results.

BMJ Open. 2020 Sep 15;10(9):e040189

Genetics & Microbiome

Microbiome Understanding in Maternity Study (MUMS), an Australian prospective longitudinal Cohort Study of Maternal and Infant Microbiota: Study Protocol

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Keywords

eNAT®	Neonatal Microbiota	Pregnancy Microbiota	Australian

Abstract

Introduction: Pregnancy induces significant physiological and cardiometabolic changes and is associated with alterations in the maternal microbiota. Increasing rates of pre-pregnancy obesity, metabolic abnormalities and reduced physical activity, all impact negatively on the microbiota causing an imbalance between the commensal microorganisms (termed dysbiosis), which may drive complications, such as gestational diabetes or hypertensive disorders. Considerable work is needed to define the inter-relationships between the microbiome, nutrition, physical activity and pregnancy outcomes. The role of the microbiota during pregnancy remains unclear. The aim of the study is to define microbiota signatures longitudinally throughout pregnancy and the first-year post birth, and to identify key clinical and environmental variables that shape the female microbiota profile during and following pregnancy.
Methods: The Microbiome Understanding in Maternity Study (MUMS) is an Australian prospective longitudinal cohort study involving 100 mother-infant pairs. Women are enrolled in their first trimester and followed longitudinally. Assessment occurs at < 13+0, 20-24 and 30-36 weeks gestation, birth and 6 weeks, 6 months and 12 months postpartum. At each assessment, self-collected oral, vaginal and faecal samples are collected using Copan eNAT® swab kit with an additional postpartum skin swab and breastmilk sample. Each infant will have oral, faecal and skin swab samples collected. Measurements include anthropometrics, body composition, blood pressure, serum hormonal and metabolic parameters and vaginal pH. Dietary intake, physical activity and psychological state will be assessed using validated self-report questionnaires, and pregnancy and infant outcomes recorded. Parametric and non-parametric hypothesis tests will be used to test the association between high-risk and low-risk pregnancies and their outcomes. This unique study will allow a comprehensive and longitudinal insight into multisite maternal and infant microbiome data using shotgun metagenomic analysis and be the first to correlate these results with rigorous metadata collected by a single research team.

JMIR Res Protoc. 2020 Jul 29;9(7):e16162

Genetics & Microbiome

Alterations of Gut Microbiota and the Brain-Immune-Intestine Axis in Patients With Relapsing-Remitting Multiple Sclerosis After Treatment with Oral Cladribine: Protocol for a Prospective Observational Study

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Keywords

eNAT®	Multiple Sclerosis	Gut Microbiota	Oral Microbiota

Abstract

Background: Immunological factors are the key to the pathogenesis of multiple sclerosis (MS). Conjointly, environmental factors are known to affect MS disease onset and progression. Several studies have found that the intestinal microbiota in MS patients differs from that of control subjects. One study found a trend toward lower species richness in patients with active disease versus in patients in remission. The microbiota plays an important role in shaping the immune system. Recent studies suggest the presence of an association between the gut microbiota and inflammatory pathways in the central nervous system.
Objective: Our goal is to examine if the changes in gut and oral microbiota and simultaneous changes in the immune response are a predictor for the treatment response in subjects with active relapsing-remitting MS (RRMS) who are being treated with oral cladribine.
Methods: This is a prospective, observational, multicenter study. Eligible subjects are patients with RRMS, between the ages of 18 and 55 years, who will start treatment with oral cladribine. Patients who used probiotics 1 month prior to the start of oral cladribine will be excluded. At baseline (ie, before start) and after 3, 12, and 24 months, the Expanded Disability Status Scale (EDSS) score will be assessed and fecal and oral samples will be collected with Copan eNAT® kit. Also, subjects will be asked to register their food intake for 7 consecutive days following the visits. After 24 months, a magnetic resonance imaging (MRI) assessment of the brain will be performed. Responders are defined as subjects without relapses, without progression on the EDSS, and without radiological progression on MRI.
Results: Inclusion started in January 2019. A total of 30 patients are included at the moment. The aim is to include 80 patients from 10 participating centers during a period of approximately 24 months. Final results are expected in 2024.
Conclusions: The results of the BIA Study will contribute to precision medicine in patients with RRMS and will contribute to a better understanding of the brain-immune-intestine axis.

J Assist Reprod Genet. 2020 Sep;37(9):2315-2326

Genetics & Microbiome

Controlled Ovarian Stimulation and Progesterone Supplementation Affect Vaginal and Endometrial Microbiota in Ivf Cycles: a Pilot Study

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Keywords

eNAT®	Controlled Ovarian Stimulation	Vaginal and Endometrial Microbiota

Abstract

Introduction: Does controlled ovarian stimulation (COS) and progesterone (P) luteal supplementation modify the vaginal and endometrial microbiota of women undergoing in vitro fertilization?
Methods: Fifteen women underwent microbiota analysis at two time points: during a mock transfer performed in the luteal phase of the cycle preceding COS, and at the time of fresh embryo transfer (ET). A vaginal swab, collected with Copan eNAT® kit, and the distal extremity of the ET catheter tip were analysed using next-generation 16SrRNA gene sequencing. Heterogeneity of the bacterial microbiota was assessed according to both the Bray-Curtis similarity index and the Shannon diversity index.
Results: Lactobacillus was the most prevalent genus in the vaginal samples, although its relative proportion was reduced by COS plus P supplementation (71.5 ± 40.6% vs. 61.1 ± 44.2%). In the vagina, an increase in pathogenic species was observed, involving Prevotella (3.5 ± 8.9% vs. 12.0 ± 19.4%), and Escherichia coli-Shigella spp. (1.4 ± 5.6% vs. 2.0 ± 7.8%). In the endometrium, the proportion of Lactobacilli slightly decreased (27.4 ± 34.5% vs. 25.0 ± 29.9%); differently, both Prevotella and Atopobium increased (3.4 ± 9.5% vs. 4.7 ± 7.4% and 0.7 ± 1.5% vs. 5.8 ± 12.0%). In both sites, biodiversity was greater after COS ($p < 0.05$), particularly in the endometrial microbiota, as confirmed by Bray-Curtis analysis of the phylogenetic distance among bacteria genera. Bray-Curtis analysis confirmed significant differences also for the paired endometrium-vagina samples at each time point.
Conclusions: Our findings suggest that COS and P supplementation significantly change the composition of vaginal and endometrial microbiota. The greater instability could affect both endometrial receptivity and placentation. If our findings are confirmed, they may provide a further reason to encourage the freeze-all strategy.

Cosmetics. 2020; 7(2):34

Genetics & Microbiome

Effects of Fermented Oils on Alpha-Biodiversity and Relative Abundance of Cheek Resident Skin Microbiota

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Keywords

eNAT®	FLOQSwabs®	Skin Microbiota	Alpha-Biodiversity

Abstract

The skin microbiome is in a very close mutualistic relationship with skin cells, influencing their physiology and immunology and participating in many dermatological conditions. Today, there is much interest in cosmetic ingredients that may promote a healthy microbiome, especially postbiotics, mainly derived from fermented products. In the present work, we studied the effects on skin microbiota of new patented natural oils obtained by unique fermentation technology in vivo. Three fermented oils were evaluated: F-Shiunko (FS), F-Artemisia® (FA) and F-Glycyrrhiza® (FG). The active components were included as single active component or in combination (FSAG) in an emulsion system. A total of 20 healthy women were recruited, and skin microbiota from cheek were analysed by mean of swab sampling (Copan FLOQSwabs® in eNAT® medium) at T0 and T1 (after 4 weeks of a one-day treatment). 16S sequencing revealed that the treatment with fermented oils improved microbiome composition and alpha-diversity. It was shown that higher biodiversity reflects in a healthier microbial ecosystem since microbial diversity decreases in the presence of a disease or due to aging. The treatment also resulted in a more "beneficial" and "younger" microbial community since a significant decrease in Proteobacteria and the increase in Staphylococcus were reported after the treatment with fermented oils.

Microbiologyopen. 2020 Jul;9(7):e1046

Genetics & Microbiome

Long-Term Stability of Microbiome Diversity and Composition in Fecal Samples Stored in eNAT® Medium

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Keywords

eNAT®	Long-Term Stability	Bacterial Inactivation

Abstract

Fecal samples collected for microbiome analyses are typically frozen to avoid post-collection changes in microbial composition. Copan eNAT® is a guanidine thiocyanate-based medium that stabilizes microbial DNA and allows safe specimen handling and shipping by inactivating microorganisms. We collected fecal samples (n = 50) from children undergoing hematopoietic stem cell transplantation. We divided samples into three aliquots: (a) stored in RNAlater and immediately transferred to –80°C; (b) stored in eNAT® medium and immediately transferred to –80°C; and (c) stored in eNAT® medium at ambient temperature (~20°C) for 30 days prior to transfer to –80°C. Mean (standard deviation) Shannon diversity and Chao1 indices in sample aliquots were 2.05 (0.62) and 23.8 (16.6), respectively. Comparing samples frozen immediately in RNA later to samples frozen immediately in eNAT®, there were no differences in Shannon diversity (p = .51), Chao1 richness (p = .66), and overall microbiome composition (p = .99). Comparing eNAT® samples frozen immediately to samples stored at ambient temperature, we identified no differences in Shannon diversity (p = .65), Chao1 richness (p = .87), and overall microbiome composition (p = .99). Storage of fecal samples in eNAT at ambient temperature for 30 days did not alter microbiome richness, diversity, or composition. eNAT® may be a useful medium for fecal microbiome studies, particularly when cold chain storage is unavailable.

Front Neurol. 2020 Nov 6;11:528056

Genetics & Microbiome

Oral Health, Oral Microbiota, and Incidence of Stroke-Associated Pneumonia - a Prospective Observational Study

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Keywords

eNAT®	Oral Microbiome	Stroke - Associated Pneumonia

Abstract

Stroke-associated pneumonia is a major cause for poor outcomes in the post-acute phase after stroke. Several studies have suggested potential links between neglected oral health and pneumonia. Therefore, the aim of this prospective observational study was to investigate oral health and microbiota and incidence of pneumonia in patients consecutively admitted to a stroke unit with stroke-like symptoms. This study involved three investigation timepoints. The baseline investigation (within 24 h of admission) involved collection of demographic, neurological, and immunological data; dental examinations; and microbiological sampling (saliva and subgingival plaque stored at -80°C in Copan eNAT® medium). Further investigation timepoints at 48 or 120 h after baseline included collection of immunological data and microbiological sampling. Microbiological samples were analyzed by culture technique and by 16S rRNA amplicon sequencing. From the 99 patients included in this study, 57 were diagnosed with stroke and 42 were so-called stroke mimics. From 57 stroke patients, 8 (14%) developed pneumonia. Stroke-associated pneumonia was significantly associated with higher age, dysphagia, greater stroke severity, embolectomy, nasogastric tubes, and higher baseline C-reactive protein (CRP). There were trends toward higher incidence of pneumonia in patients with more missing teeth and worse oral hygiene. Microbiological analyses showed no relevant differences regarding microbial composition between the groups. However, there was a significant ecological shift over time in the pneumonia patients, probably due to antibiotic treatment. This prospective observational study investigating associations between neglected oral health and incidence of SAP encourages investigations in larger patient cohorts and implementation of oral hygiene programs in stroke units that may help reducing the incidence of stroke-associated pneumonia.

Heliyon. 2021 Mar 18;7(3):e06513

Genetics & Microbiome

Temporal Oral Microbiome Changes with Brushing in Children with Cleft Lip and Palate

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Keywords

FLOQSwabs®	Oral Microbiome	Cleft Lip and Palate

Abstract

This cohort study aimed to characterize the oral microbiome of children with CLP, from two different age groups, and evaluate the effect of supervised or unsupervised toothbrushing on the microbiome of the cleft over time. Copan FLOQSwabs® oral swab samples were collected from the cleft area at three different time points (A; no brushing, B; after 15 days and C; after 30 days) and were analyzed using next-generation sequencing to determine the microbial composition and diversity in these time points. Overall, brushing significantly decreased the abundance of the genera Alloprevotella and Leptotrichia in the two age groups examined, and for Alloprevotella this decrease was more evident for children (2–6 years old). In the preteen group (7–12 years old), a significant relative increase of the genus Rothia was observed after brushing. In this study, the systematic brushing over a period of thirty days also resulted in differences at the intra-individual bacterial richness.

Crit Care Explor. 2021 Mar 17;3(3):e0360

Genetics & Microbiome

Decreased Intestinal Microbiome Diversity in Pediatric Sepsis: a Conceptual Framework for Intestinal Dysbiosis to Influence Immunometabolic Function

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Keywords

FLOQSwabs®	GUT Microbiome	Pediatric Sepsis

Abstract

The intestinal microbiome can modulate immune function through production of microbial-derived short-chain fatty acids. We explored whether intestinal dysbiosis in children with sepsis leads to changes in microbial-derived short-chain fatty acids in plasma and stool that are associated with immunometabolic dysfunction in peripheral blood mononuclear cells. We enrolled Forty-three children with sepsis/septic shock and 44 healthy controls. Stool (collected with Copan FLOQSwabs®) and plasma samples were serially collected for sepsis patients; stool was collected once for controls. The intestinal microbiome was assessed using 16S ribosomal RNA sequencing and alpha- and beta-diversity were determined. We measured short-chain fatty acids using liquid chromatography, peripheral blood mononuclear cell mitochondrial respiration using high-resolution respirometry, and immune function using ex vivo lipopolysaccharide-stimulated whole blood tumor necrosis factor-α. Sepsis patients exhibited reduced microbial diversity compared with healthy controls, with lower alpha- and beta-diversity. Reduced microbial diversity among sepsis patients (mainly from lower abundance of commensal obligate anaerobes) was associated with increased acetic and propionic acid and decreased butyric, isobutyric, and caproic acid. Decreased levels of plasma butyric acid were further associated with lower peripheral blood mononuclear cell mitochondrial respiration, which in turn, was associated with lower lipopolysaccharide-stimulated tumor necrosis factor-α. However, neither intestinal dysbiosis nor specific patterns of short-chain fatty acids were associated with lipopolysaccharide-stimulated tumor necrosis factor-α. Intestinal dysbiosis was associated with altered short-chain fatty acid metabolites in children with sepsis, but these findings were not linked directly to mitochondrial or immunologic changes. More detailed mechanistic studies are needed to test the role of microbial-derived short-chain fatty acids in the progression of sepsis.

Crit Care Explor. 2021 Mar 17;3(3):e0360

Genetics & Microbiome

Detection of Microbial Agents in Oropharyngeal and Nasopharyngeal Samples of SARS-CoV-2 Patients

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Keywords

FLOQSwabs®	Oro- Nasopharyngeal Microbiome	SARS-CoV-2 Infection

Abstract

The novel coronavirus outbreak started in December 2019 and rapidly spread around the globe, leading to a global pandemic. Here we reported the association of microbial agents identified in oropharyngeal and nasopharyngeal samples collected with Copan FLOQSwabs®, from patients with SARS-CoV-2 infection, using a Pan-microarray based technology referred to as PathoChIP. To validate the efficiency of PathoChIP, reference viral genomes obtained from BEI resource and 25 SARS-CoV-2 positive clinical samples were tested. This technology successfully detected femtogram levels of SARS-CoV-2 viral RNA, which demonstrated greater sensitivity and specificity than conventional diagnostic techniques. Simultaneously, a broad range of other microorganisms, including other viruses, bacteria, fungi and parasites can be detected in those samples. We identified 7 viral, 12 bacterial and 6 fungal agents common across all clinical samples suggesting an associated microbial signature in individuals who are infected with SARS-CoV-2. This technology is robust and has a flexible detection methodology that can be employed to detect the presence of all human respiratory pathogens in different sample preparations with precision. It will be important for differentiating the causative agents of respiratory illnesses, including SARS-CoV-2.

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Genetics & Microbiome

Detection of Cell-Free Foetal DNA Fraction in Female-Foetus Bearing Pregnancies Using X-Chromosomal Insertion/Deletion Polymorphisms Examined by Digital Droplet PCR

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Keywords

hDNAFLOQSwabs®	Genetics	X-chromosomal INDEL	ddPCR

Abstract

In families with X-linked recessive diseases, foetal sex is determined prenatally by detection of Y-chromosomal sequences in cell-free foetal DNA (cffDNA) in maternal plasma. The same procedure is used to confirm the cffDNA presence during non-invasive prenatal RhD incompatibility testing but there are no generally accepted markers for the detection of cffDNA fraction in female-foetus bearing pregnancies. We present a methodology allowing the detection of paternal X-chromosomal alleles on maternal background and the confirmation of female sex of the foetus by positive amplification signals. Using digital droplet PCR (ddPCR) we examined X-chromosomal INDEL (insertion/deletion) polymorphisms: rs2307932, rs16397, rs16637, rs3048996, rs16680 in buccal swabs (Copan Italia, Brescia) of 50 females to obtain the population data. For all INDELs, we determined the limits of detection for each ddPCR assay. We examined the cffDNA from 63 pregnant women bearing Y-chromosome negative foetuses. The analysis with this set of INDELs led to informative results in 66.67% of examined female-foetus bearing pregnancies. Although the population data predicted higher informativity (74%) we provided the proof of principle of this methodology. We successfully applied this methodology in prenatal diagnostics in a family with Wiscott–Aldrich syndrome and in pregnancies tested for the risk of RhD incompatibility.

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Genetics & Microbiome

Biotechnical Development of Genetic Addiction Risk Score (GARS) and Selective Evidence for Inclusion of Polymorphic Allelic Risk in Substance Use Disorder (SUD)

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Keywords

4N6FLOQSwabs®	Buccal Cell	Genetic Rick Score

Abstract

Research into the neurogenetic basis of addiction identified and characterized by Reward Deficiency Syndrome (RDS) includes all drug and non-drug addictive, obsessive and compulsive behaviours. We are proposing herein that a new model for the prevention and treatment of Substance Use Disorder (SUD) a subset of RDS behaviors, based on objective biologic evidence, should be given serious consideration in the face of a drug epidemic. The development of the Genetic Addiction Risk Score (GARS) followed seminal research in 1990, whereby, Blum's group identified the first genetic association with severe alcoholism published in JAMA. While it is true that no one to date has provided adequate RDS free controls there have been many studies using case –controls whereby SUD has been eliminated. We argue that this deficiency needs to be addressed in the field and if adopted appropriately many spurious results would be eliminated reducing confusion regarding the role of genetics in addiction. However, an estimation, based on these previous literature results provided herein, while not representative of all association studies known to date, this sampling of case- control studies displays significant associations between alcohol and drug risk. In fact, we present a total of 110,241 cases and 122,525 controls derived from the current literature. We strongly suggest that while we may take argument concerning many of these so-called controls (e.g. blood donors) it is quite remarkable that there are a plethora of case –control studies indicating selective association of these risk alleles (measured in GARS) for the most part indicating a hypodopaminergia. The paper presents the detailed methodology of the GARS. Data collection procedures, sample collection with Copan 4N6FLOQSwab®, instrumentation, and the analytical approach used to obtain GARS and subsequent research objectives are described.

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Genetics & Microbiome

2.7 Million Samples Genotyped for HLA by Next Generation Sequencing: Lessons Learned

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Keywords

hDNAFLOQSwabs®	Self-Administered Swabs	High Throughput	NGS

Abstract

Background: At the DKMS Life Science Lab, Next Generation Sequencing (NGS) has been used for ultra-high- volume high-resolution genotyping of HLA loci for the last three and a half years. Here, we report on our experiences in genotyping the HLA, CCR5, ABO, RHD and KIR genes using a direct amplicon sequencing approach on Illumina MiSeq and HiSeq 2500 instruments.
Results: Between January 2013 and June 2016, 2,714,110 samples largely from German, Polish and UK-based potential stem cell donors have been processed. 98.9% of all alleles for the targeted HLA loci (HLA-A, -B, -C, -DRB1, -DQB1 and -DPB1) were typed at high resolution or better. Initially a simple three-step workflow based on nanofluidic chips in conjunction with 4-primer amplicon tagging was used. Over time, we found that this setup results in PCR artefacts such as primer dimers and PCR-mediated recombination, which may necessitate repeat typing. Split workflows for low- and high-DNA-concentration samples helped alleviate these problems and reduced average per-locus repeat rates from 3.1 to 1.3%. Further optimisations of the workflow included the use of phosphorothioateoligos to reduce primer degradation and primer dimer formation, and employing statistical models to predict read yield from initial template DNA concentration to avoid intermediate quantification of PCR products. Finally, despite the populations typed at DKMS Life Science Lab being relatively homogenous genetically, an analysis of 1.4 million donors processed between January 2015 and May 2016 led to the discovery of 1,919 distinct novel HLA alleles.
Conclusions: Amplicon-based NGS HLA genotyping workflows have become the workhorse in high-volume tissue typing of registry donors. The optimisation of workflow practices over multiple years has led to insights and solutions that improve the efficiency and robustness of short amplicon based genotyping workflows.

Who is DKMS?

DKMS is an international nonprofit organization dedicated to the fight against blood cancer and blood disorders by creating awareness, recruiting bone marrow donors to provide a second chance at life and raising funds to match donor registration costs. With over 8 million potential donors registered worldwide, since 2014 DKMS embraced Copan hDNAFree FLOQSwabs® collection system to find compatible donors for stem cell transplantation.

Mol Cytogenet. 2019 Nov 21;12:48

Genetics & Microbiome

Targeted Capture Enrichment Followed by NGS: Development and Validation of a Single Comprehensive NIPT for Chromosomal Aneuploidies, Microdeletion Syndromes and Monogenic Diseases

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Keywords

hDNAFreeFLOQSwabs®	Chromosomal Aneuploidies	NGS	Monogenic Diseases

Abstract

Background: Non-invasive prenatal testing (NIPT) has been widely adopted for the detection of fetal aneuploidies and microdeletion syndromes, nevertheless, limited clinical utilization has been reported for the non-invasive prenatal screening of monogenic diseases. In this study, we present the development and validation of a single comprehensive NIPT for prenatal screening of chromosomal aneuploidies, microdeletions and 50 autosomal recessive disorders associated with severe or moderate clinical phenotype.
Results: We employed a targeted capture enrichment technology powered by custom TArget Capture Sequences (TACS) and multi-engine bioinformatics analysis pipeline to develop and validate a novel NIPT test. This test was validated using 2033 cell-fee DNA (cfDNA) samples from maternal plasma of pregnant women referred for NIPT and paternal genomic DNA extracted starting from hDNAfree buccal swab (Copan Italia, Brescia). Additionally, 200 amniotic fluid and CVS samples were used for validation purposes. All NIPT samples were correctly classified exhibiting 100% sensitivity (CI 89.7–100%) and 100% specificity (CI 99.8–100%) for chromosomal aneuploidies and microdeletions. Furthermore, 613 targeted causative mutations, of which 87 were unique, corresponding to 21 monogenic diseases, were identified. For the validation of the assay for prenatal diagnosis purposes, all aneuploidies, microdeletions and point mutations were correctly detected in all 200 amniotic fluid and CVS samples.
Conclusions: We present a NIPT for aneuploidies, microdeletions, and monogenic disorders. To our knowledge this is the first time that such a comprehensive NIPT is available for clinical implementation.

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Genetics & Microbiome

Validation of Genome-Wide Association Study-Identified Single Nucleotide Polymorphisms in a Case-Control Study of Pancreatic Cancer from Taiwan

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Keywords

hDNAFLOQSwabs®	Genome Wide-Association	Pancreatic Cancer

Abstract

Background: Due to differences in genetic background, it is unclear whether the genetic loci identified by the previous genome-wide association studies (GWAS) of pancreatic cancer also play significant roles in the development of pancreatic cancer among the Taiwanese population.
Methods: This study aimed to validate the 25 pancreatic cancer GWAS-identified single nucleotide polymorphisms (SNPs) starting from Buccal Swab extracted DNA (Copan Italia, Brescia) in a case-control study (278 cases and 658 controls) of pancreatic cancer conducted in Taiwan. Statistical analyses were conducted to determine the associations between the GWAS-identified SNPs and pancreatic cancer risk. Gene-environment interaction analysis was conducted to evaluate the interactions between SNPs and environmental factors on pancreatic cancer risk.
Results: Among the 25 GWAS-identified SNPs, 7 (rs2816938 (~ 11 kb upstream of NR5A2), rs10094872 (~ 28 kb upstream of MYC), rs9581943 (200 bp upstream of PDX1) and 4 chromosome 13q22.1 SNPs: rs4885093, rs9573163, rs9543325, rs9573166) showed a statistically significant association with pancreatic cancer risk in the current study. Additional analyses showed two significant gene-environment interactions (between poor oral hygiene and NR5A2 rs2816938 and between obesity and PDX1 rs9581943) on the risk of pancreatic cancer.
Conclusions: The current study confirmed the associations between 7 of the 25 GWAS-identified SNPs and pancreatic risk among the Taiwanese population. Furthermore, pancreatic cancer was jointly influenced by lifestyle and medical factors, genetic polymorphisms, and gene-environment interaction. Additional GWAS is needed to determine the genetic polymorphisms that are more relevant to the pancreatic cancer cases occurring in Taiwan.

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Genetics & Microbiome

Early Origins of Allergy and Asthma (AIRES): Study Protocol for a Prospective Prenatal Birth Cohort in Chile

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Keywords

hDNAFreeFLOQSwabs®	Allergy and Asthma	Genetics	Epigenetic Factors

Abstract

Background: Growing evidence shows that atopic dermatitis (AD), food allergy (FA), allergic rhinitis, and asthma are largely determined during the first 1000 days (time elapsed from conception to the 2nd birthday). The ARIES birth cohort aims to determine prenatal and perinatal conditions, as well as genetic and epigenetic factors, that participate in the early setting of immune responses, and the role of these in the later determination of the risk of allergic diseases and asthma in the offspring. Methods: We have designed a birth cohort of 250 families with prenatal recruitment (~ 14 weeks). We have genotyped relevant allergy/asthma-associated variants in trios and performed immunophenotyping starting from buccal swab (Copan Italia, Brescia) and evaluation of allergy biomarkers in cord blood. At 1 and 2 years of age we have assessed if infants have developed allergic sensitization, AD, FA, as well as biomarkers of asthma including the asthma predictive index. We have also evaluated how maternal conditions modify immune programming through epigenetic modifications and we have then depicted newborn epigenetic cues of allergy/asthma risk. Next, we have assessed composition/diversity of maternal gut, placenta, breastmilk and infant gut microbiome and their association with immunophenotype and biomarkers at birth, and clinical outcomes at age 1 and 2. Finally, we plan to assess how environmental exposures (perinatal outdoor and indoor pollution, allergens and endotoxin) affect the incidence of allergic sensitization, AD, FA, and risk of asthma. Discussion: The in-depth study of the ARIES birth cohort shall provide crucial information to understand the rising incidence of allergies and asthma in developing countries, and hopefully provide cues on how to prevent and treat these diseases.

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Genetics & Microbiome

Noninvasive Prenatal Paternity Testing by Means of SNP-Based Targeted Sequencing

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Keywords

hDNAFLOQSwabs®	Prenatal Paternity Testing	Single Nucleotide Polymorphisms

Abstract

Objective: To develop a method for noninvasive prenatal paternity testing based on targeted sequencing of single nucleotide polymorphisms (SNPs).
Method: SNPs were selected based on population genetics data. Target-SNPs in cellfree DNA extracted from maternal blood (maternal cfDNA) and paternal buccal sample (Copan Buccal Swab) were analyzed by targeted sequencing wherein target enrichment was based on multiplex amplification using QIAseq Targeted DNA Panels with Unique Molecular Identifiers. Fetal SNP genotypes were called using a novel bioinformatics algorithm, and the combined paternity indices (CPIs) and resultant paternity probabilities were calculated.
Results: Fetal SNP genotypes obtained from targeted sequencing of maternal cfDNA were 100% concordant with those from amniotic fluid-derived fetal genomic DNA. From an initial panel of 356 target-SNPs, an average of 148 were included in paternity calculations in 15 family trio cases, generating paternity probabilities of greater than 99.9999%. All paternity results were confirmed by short-tandem-repeat analysis. The high specificity of the methodology was validated by successful paternity discrimination between biological fathers and their siblings and by large separations between the CPIs calculated for the biological fathers and those for 60 unrelated men.
Conclusion: The novel method is highly effective, with substantial improvements over similar approaches in terms of reduced number of target-SNPs, increased accuracy, and reduced costs.

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Genetics & Microbiome

High-Throughput MICA/B Genotyping of Over Two Million Samples: Workflow and Allele Frequencies

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Keywords

hDNAFreeFLOQSwabs®	MICA and MICB	Hematopoietic Transplantation

Abstract

MICA and MICB are ligands of the NKG2D receptor and thereby influence NK and T cell activity. MICA/B gene polymorphisms, expression levels and the amount of soluble MICA/B in the serum have been linked to autoimmune diseases, infections, and cancer. In hematopoietic stem cell transplantation, MICA matching between donor and patient has been correlated with reduced acute and chronic graft-vs.-host disease and improved survival. Hence, we developed an extremely cost-efficient high-throughput workflow for genotyping MICA/B for newly registered potential stem cell donors. Since mid-2017, we have genotyped over two million samples using NGS amplicon sequencing for MICA/B exons 2–5 starting from DNA collected with buccal swab (Copan Italia, Brescia). In donors of German origin, MICA*008 is the most common MICA allele with a frequency of 42.3%. It is followed by MICA*002 (11.7%) and MICA*009 (8.8%). The three most common MICB alleles are MICB*005 (43.9%), MICB*004 (21.7%), and MICB*002 (18.9%). In general, MICB is less diverse than MICA and only 6 alleles, instead of 15, account for a cumulative allele frequency of 99.5%. In 0.5% of the samples we observed at least one allele of MICA or MICB which has so far not been reported to the IPD/IMGT-HLA database. By providing MICA/B typed voluntary donors, clinicians become empowered to include MICA/B into their donor selection process to further improve unrelated hematopoietic stem cell transplantation.

Note

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