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Title: Characterizing and/or identifying different microorganism species from test sample, by obtaining test sample, lysing non-microorganism cells in test sample to produce lysed sample; layering test or lysed sample on density cushion

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Abstract: NOVELTY - The method comprises: obtaining a test sample known to contain or that may contain different microorganism species; optionally selectively lysing non-microorganism cells in the test sample to produce a lysed sample; layering the test or lysed sample on a density cushion in a sealed container; centrifuging the container to separate the microorganism species from other components the sample and form a mixed pellet the microorganism species, where the microorganism species form enriched layers within the pellet; and spectroscopically interrogating each the enriched layers.

USE - The method is useful for characterizing and/or identifying different microorganism species from a test sample including blood, urine, feces, intravenous catheters, industrial production lines, water systems, a food product, a cosmetic product, a pharmaceutical product and a forensic sample.

ADVANTAGE - The method is capable simply, rapidly, safely, reliably and effectively characterizing and/or identifying different microorganism species from the test sample.

DETAILED DESCRIPTION - The method comprises: obtaining a test sample known to contain or that may contain different microorganism species; optionally selectively lysing non-microorganism cells in the test sample to produce a lysed sample; layering the test or lysed sample on a density cushion in a sealed container; centrifuging the container to separate the microorganism species from other components the sample and form a mixed pellet the microorganism species, where the microorganism species form enriched layers within the pellet; spectroscopically interrogating each the enriched layers the microorganism species in the mixed pellet to produce spectroscopic measurements the microorganism species; and characterizing and/or identifying each the microorganism species in the enriched layers the mixed pellet by comparison the spectroscopic measurements with spectroscopic measurements taken or spectroscopic properties predicted known microorganisms. The interrogation step is non-invasive. The spectroscopic interrogation the enriched layers microorganisms is carried through an optical window located on the side the separation container. The spectroscopic measurements are present from fluorescence spectroscopy and Raman spectroscopy. The spectroscopy is fluorescence spectroscopy, diffuse reflectance spectroscopy, absorption and transmission spectroscopy, infrared spectroscopy, terahertz spectroscopy, Raman spectroscopy, surface enhanced Raman Spectroscopy, spatially fset Raman spectroscopy, transmission Raman spectroscopy and/or resonance Raman spectroscopy. The fluorescence spectroscopy is measured in front face mode. The identification is based on intrinsic fluorescence the microorganism. The spectroscopy comprises determining an excitation-emission matrix (EEM). The EEM is compared to a database EEMs known microorganisms. The microorganisms are characterized based on: phenotypic and/or morphologic characteristics; and measurements detection time, growth rate and microorganism pellet size, shape, color and/or density. The microorganisms are characterized

into classification models consisting gram groups, clinical gram groups, therapeutic groups, functional groups, and natural intrinsic fluorescence groups. The microorganisms are identified to the genus level, species level, or strain level. The step lysing non-microorganism cells is performed by sonication, osmotic shock and/or chemical treatment, and a lysis solution comprising detergents, which are Triton X-100 (RTM: Nonionic surfactant), TritonX-100-R (RTM: Polyethylene glycol p-(1,1,3,3-tetramethylbutyl)-phenyl ether), Triton X-114 (RTM: Octylphenol ethoxylate surfactant), nonyl phenoxypolyethoxylethanol, Genapol C-100 (RTM: Isotridecanol polyglycol ether), Genapol X-100 (RTM: Isotridecanol polyglycol ether), Igepal CA 630 (RTM: Octylphenoxypolyethoxyethanol), Arlasolve 200 (RTM: Polyoxyethylene 20 isohexadecylether), 3-((3-cholamidopropyl)dimethylammonium)-1- propanesulfonate, octyl p-D-glucopyranoside, saponin, nonaethylene glycol monododecyl ether (C12E9, polidocenol), sodium dodecyl sulfate, N-laurylsarcosine, sodium deoxycholate, bile salts, hexadecyltrimethylammonium bromide, 3-(Decyldimethylazaniumyl)propane-1-sulfonate, SB3-12, amidosulfobetaine-14, 3-(4-heptyl) phenyl 3-hydroxy propyl dimethyl ammonio propane sulfonate, Brij 98 (RTM: Polyoxyethylene-(20)-oleyl-ether), Brij 58 (RTM: Polyoxyethylene-(20)-cetyl-ether), Brij 35 (RTM: Polyoxyethylene-(23)-lauryl-ether), Tween 80 (RTM: Polyoxyethylene sorbitan monooleate), Tween 20 (RTM: Polyoxyethylene sorbitan monolaurate), Pluronic L64 (RTM: Block copolymer surfactant), Pluronic P84 (RTM: block copolymer surfactant), NDSB 201 (RTM: Non-detergent sulfobetaines) , amphipols (PMAL-C8), and methyl-P-cyclodextrin. The detergent is a polyoxyethylene detergent comprising the structure 12-18C/E9-10. The polyoxyethylene detergent is Brij 97 (RTM: Polyoxyethylene-(10)-oleyl-ether), Genapol C-100 (RTM: Isotridecanol polyglycol ether), Genapol X-100 (RTM: Isotridecanol polyglycol ether), and polidocenol. The lysis solution further comprises: enzymes comprising a mixture proteinases and/or nucleases; and buffering agents. The density cushion comprises colloidal silica, iodinated contrast agents, sucrose, microscope immersion oil, mineral oil, silicone oil, fluorosilicone oil, silicone gel, metrizoate-Ficoll, diatrizoate-dextran, carboxymethyl cellulose, hydroxypropylmethyl cellulose, polyethylene oxide (high molecular weight), Pluronic F68 (RTM: Block copolymers based on ethylene oxide and propylene oxide), Pluronic F-127 (RTM: Nonionic, surfactant polyol), polyacrylic acid, cross-linked polyvinyl alcohol, cross-linked polyvinyl pyrrolidine, PEG methyl ether methacrylate, pectin, agarose, xanthan, gellan, Phytagel (RTM: Gelling agent), sorbitol, Ficoll (RTM: neutral, highly branched, high-mass, hydrophilic polysaccharide), glycerol, dextran, glycogen, cesium chloride, perfluorocarbon fluids, and/or hydrluorocarbon fluid. The test sample is a culture sample known to contain microorganism species. The visual determination the presence a mixed culture and/or separate or distinct layers a mixed culture, are aided with the use selective dyes.

Drawing:

Derwent Class Code(s): A89 (Photographic, laboratory equipment, optical); D16 (Fermentation industry); B04 (Natural products and polymers, testing, compounds unknown structure); D13 (Other foodstuffs and treatment); D15 (Treating water, industrial waste and sewage); D21 (Preparations for dental or toilet purposes); S03 (Scientific Instrumentation, photometry, calorimetry); T01 (Digital Computers)

Derwent Manual Code(s): A12-L04B; A12-V03C2; A12-W11L; D03-H01T2B; D03-K03; D03-K04; D04-A01; D05-H04; D05-H05; D05-H06A; D08-B; B04-B01C3; B04-C02; B04-F01; B04-L05A; B04-L05C; B11-C07B; B11-C08A; B11-C08D3; B11-C11; B12-K04A; S03-E13D;

S03-E14H; T01-C07C2; T01-J05B2; T01-J05B4P
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Compound(s):

DCR Number	Role	DCR Number	Role	DCR Number	Role
92818-0-0-0	K; M	133912-0-0-0	K; M	798399-1-0-0	K; M
114270-0-0-0	K; M	103468-0-0-0	K; M	86730-0-0-0	K; M
2418795-0-0-0	K; M				

Derwent Compound Number(s):

Compound Number	Role	Compound Number	Role	Compound Number	Role
R01857	K; M	R16573	K; M	R01835	K; M
R06717	K; M	RAC4FV	K; M	RA03GS	K; M
R17032	K; M	RA05MC	K; M	RBAAY7	K; M

Derwent Registry Number(s):

Registry Number	Role	Registry Number	Role
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