Hellenic Accreditation System

Annex G1/15 to the Certificate No.**715**-5

SCOPE of ACCREDITATION

for the

Clinical Laboratory

of

“ΑΛΦΑ LAB Molecular Biology and Cytogenetics Center S.A.”

Tested materials/ products	Types of test/ Properties to be measured	Applied Standards/ Techniques to be used
Cytogenetics		
1. Bone Marrow , Peripheral Blood , Lymphatic tissue	1. Karyotype /Detection of acquired chromosome abnormalities	*In house methods (Π.Κ.01v.3-2/1/2013, Π.Κ.02v.4-22/6/2015, Π.Κ.03v.3-22/8/2019, Π.Κ.04v.5-12/12/2017, Π.Κ.05v.4-1/6/2015).*Cells culturing, cell collection (harvest), and karyotyping (Chromosome analysis) based on international bibliography according to ACC and ECA Cytogenetic Guidelines and Quality Assurance, 2005 and ISCN, 2013.
2. Peripheral Blood	2. Karyotype /Detection of chromosome abnormalities	
3. Amniotic fluid		
4. Chorionic villus sample (CVS)		
5. Products of conception and samples of abortuces		
Molecular Genetics		
1. Amniotic fluid, Chorionic villus sample (CVS), Products of conception and samples of abortuces	1. QF PCR and detection of 508del mutation of the cystic fibrosis gene.	*In house methods (Π.Μ.04v4-14/5/2018).* Polymerase Chain Reaction (PCR) with the use of STRs on specific sites of chromosomes 13, 18, 21, X and Y. For this protocol we use PCR BIORAD t100 (M.2.26) Fragment analysis and quantitative estimate of the PCR products with the use of ABI 3500, ABI 3130XL (s/n 24163-171, 21270-004) and analysis program GeneMapper ID.
2. Paraffin-embedded tissues	1. Mutation detection in KRAS gene (exons 2,3,4)	Protocols Π.Μ.11v.2-26/1/2017, Π.Μ.26v.2-26/1/2017, Π.Μ.19v.2-26/1/2017. For these protocols we use PCR BIORAD t100(M.2.22) Fragm

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	2.Mutation detection in NRAS gene (exons 2,3,4)	Determination of DNA sequences with the use of Big Dye Terminator kit RUO v.1.1 (ABI) and the analysis of the results in genetic analyzer ABI 3500 and ABI 3130XL (s/n 24163-171, 21270-004).
	3. Mutation detection in C-KIT gene (exons 9,11,13,17)	
	4. Mutation detection in EGFR gene (exons 18,19,20,21)	Protocols Π.Μ.18v.3-26/1/2017, Π.Μ.27v.2-26/1/2017, Π.Μ.20v.2-26/1/2017. For these protocols we use PCR BIORAD t100 (M.2.22). Determination of DNA sequences with the use of Big Dye Terminator kit RUO v.1.1 (ABI) and the analysis of the results in genetic analyzers ABI 3500 and ABI 3130XL (s/n 24163-171, 21270-004).
	5.Mutation detection in BRAF gene (exons 11,15)	
	6. Mutation detection in PDGFR gene (exons 12,14,18)	
3. Peripheral Blood and paraffin-embedded tissues	1. Mutation detection in BRCA1 & BRCA2 genes associated with Breast and Ovarian cancer.	Next Generation Sequencing (NGS) using CLEANEX BRCA1, BRCA2 v3 PARAGONE (RUO) kit (Protocols Π.Μ-56 and Π.Μ.-59). For the libraries we use PCR BIORAD T100 (M.3-2.25) Statistical analysis is performed with Varsome Clinical software.
4. Peripheral Blood	Variant detection in 37 genes- BRCA1, BRCA2, ATM, BARD1, BLM, BRIP1, CDH1, CHEK2, EPCAM, FAM175A, MEN1, MRE11A, MSH2, MSH6, MLH1, MUTYH, NBN, PALB2, PMS2, PMS2CL, PTEN, RAD50, RAD51C, RAD51D, STK11, TP53, XRCC2, APC, RNF139,MUTF, SMAD4, BAP1, CDK4, POLD1, POLE, CDKN2A, BMPR1A, GREM1 - associated with breast/ovarian cancer as well as other types of cancer.	Next Generation Sequencing (NGS) using CLEANPLEX HEREDITARY CANCER PANEL v2 PARAGONE kit (Protocols Π.Μ-57 and Π.Μ.-59). For the libraries, we use PCR BIORAD T100 (M.3-2.25). Bioinformatics is performed with Varsome Clinical software
Molecular microbiology		
1. Paraffin-embedded tissues, tissue sample from biopsy, cellular swab.	Detection and genotyping of Human Papilloma Virus (HP*V*)- 41 τύποι (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 26, 34, 53, 66, 67, 68a 68b, 69, 70, 73, 82(IS39, 6, 11, 40, 42, 43, 44, 54, 55, 57, 61, 62, 72, 81(CP8304), 83(MM7), 84(MM8), 90, 91.82(MM4)	Protocol Π.Μ.54 VISION ARRAY HPV DETECTION KIT by MENARINI (CE IVD). Hybridization is performed by TDH-500 Denaturation and Hybridization System (M.Hybridizer.1). analysis of the results with Vision *Array* Analysis Package MultiScan (M.Vision Array.1) QIAamp DNA blood mini kit by Qiagen, for DNA extraction. Thermal cycler: BIORAD T100 (M.3-2.24)

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2.Oropharyngeal swab and nasopharyngeal swab	Molecular detection of RNA of Sars-Cov-2 Virus.	Π.Μ.52v.1-2020 Molecular detection of Sars-Cov-2 with real time PCR in Ari-aDX (Agilent) (s/n MY20435332, MY20435308, MY20345323), with YOUSEQ COVID-19 2 GENE MULTI-PLEX qRT-PCR KIT(CE IVD).
3.Ball, sputum, lung paracentesis sample etc.	Molecular detection of DNA of Mycobacterium tuberculosis complex with Real-Time PCR	Π.Μ.58v.1 Molecular detection of MTB complex with real time PCR, us-ing TRU PCR MTBC one step Nested kit (CE IVD) of BLACK BIO. Thermal cyclers AriaDX (Agilent) (s/n MY20435332, MY20435308, MY20345323).
Molecular Cytogenetics		
1. Bone marrow, Peripheral Blood, Paraffin-embedded tissues (section), surgical biopsy.	Florescent In Situ Hybridization (FISH), with DNA probes for the detection of: 1. Chromosome aneuploidies, [+8,+9,+12,+15], 2. Fusion genes (BCR-ABL, ETO-AML1,TEL-AML1, PML-RARA,IgH-CCND1, IgH-FGFR3, IgH- BCL2,IgH-MAF,CBFβ/MYH11, FIP1L1/ PDGFRA,IGH/MAF3, TCF3/PBX1), 3. Gene rearrangements (IgH,MLL,AML1,PDGFRB,TCRa/d, c-MYC,IGL,IGK,BCL6,FGFR1, MALT1), 4. Chromosome / Gene deletions (5/5q, 7/7q, P16(9p21), -13/13qdel, P53(17p), ATM(11q23),del(20q), MYB(6q23),1p32, 5. Gene amplifications (1q21).	*In house methods* Protocol Π.Κ.10v.1-2/1/2014, Π.Κ.10Bv.1-2/1/2014 *In Situ* Hybridization with DNA probes for specific or repeated sequences. In situ hybridization with THERMOBRITE thermal cycler (s/n 507S50000194).
2. Amniotic fluid, Chorionic villus sample (CVS), Products of conception and samples of abortuces	Prenatal screening with Array Comparative genomic Hybridization using Agilent's SurePrint G3 Human CGH Microarray 8X60K platform (Prenatal Molecular Karyotype)	Protocol Π.Κ.11v.1-1/1/2018 Agilent Oligonucleotide Array-Based CGH gor 8x60K design (SurePrint G3 Human CGH ISCA v2 Microarray 8×60K) System analysis Agilent CytoGenomics software with Agilent Technologies DNA Microarray Scanner (s/n G2505C)
3. Peripheral Blood	Postnatal screening with Array Comparative genomic Hybridization using Agilent SurePrint G3 Human CGH+SNP Microarray 4X180K platform (Postnatal Molecular Karyotype)	Protocol Π.Κ.12v.1-1/1/2018 Agilent Oligonucleotide Array-Based CGH gor 4x180K+SNPsdesign (SurePrintG3 Human ISCA CGH + SNP Microarray 4×180K) System analysis Agilent CytoGenomics software with Agilent Technologies

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		DNA Microarray Scanner (s/n G2505C)
4. Blastomere, Trophoectoderm, Polar body	Pre-Implantation Genetic screening for chromosomal Aneuploidy (PGD/PGS) 1. using Agilent Pre-screen Microarray, 8X60K Platform (Array-CGH) 2. using VeriSeq Illumina Platform (NGS)	Protocol Π.Κ.13v.1-1/1/2015 Agilent GenetiSure Pre-Implantation Array-Based CGH for Aneuploidy Screening GenetiSure Pre-Screen Microarray, 8×60K. System analysis Agilent CytoGenomics software with Agilent Technologies DNA Microarray Scanner (s/n G2505C) Protocol Π.Μ.43Αv.1-1/1/2022 Thermo Fiscer Protoseq (CE-IVD) Pre-Implantation NGS-Based screening for chromosomal Aneuploidy (PGS) with NGS technology. Analyzer Ion Torrent , Thermo Fiscer (M.I-TOR1) Analysis with Ion Torrent Cloud Reporter software
5. Blastomere, Trophoectoderm, Polar body	Pre-Implantation Genetic Diagnosis of variants in b-globin gene (PGD b-thalassemia)	Protocol Π.Μ.42v.1-1/1/2018, Π.Μ.49v.1-1/1/2018 For these protocols we use PCR BIORAD t100(M.2.25) SNaPshot® Multiplex System STR multiplex PCR RUO 3130xl ABI GeneScan Analysis Software version 3.5 (s/n 21270-004), 3500 ABI sequencer GeneScan. Analysis Software version 4.1 (s/n 24163-171).
6. Peripheral blood, amniotic fluid, CVS	Variant detection in CFTR gene with method NGS	Protocols Π.Μ.55v.1 - Π.Μ.46v.1 -CLEANPLEX CUSTOM PANEL PGD 14 v2 of PARAGONE. For the libraries we use PCR BIORAD T100 (M.3-2.25) MiSeq – Illumina (s/n 1067-12-7576) NGS data analysis with SeqPilot JSI software.

Site of assessment: **Laboratory permanent premises: 11 Anastasiou str., GR-115 24, Athens, Greece.**
Branch – sampling site: **ICEBOX backyard of laboratory permanent premises.

Approved signatories**: N.Spanakis, L.Florentin, F.Sachinidi, C.Bili, Th.Varvouni, A.Maravelaki, E.Panou, E.Manisali, J.Donoghue-Nadali, S.Christopoulou, E.Vrettou, S.Papadopoulou, N.Pisimisis.**

The Accreditation Certificate No. **715-**5, to ELOT EN ISO 15189:2012, is valid until 08.11.2027.

Athens, 02.02.2023

Christos Nestoras
CEO of ESYD