Neisseria gonorrhoeae

Background: Neisseria gonorrhoeae is a gram negative oxidase positive bacteria producing a lipooligosaccharide endotoxin which contains lipid A without long repeating sugar side chains. Pili for attachment to mucosal cell surfaces and antiphagocytic action and IgA proteases are further important virulence factors. Antigen and outer membrane protein changes enable N. gonorrhoeae to cause repeated infection. Disseminated infection is due in part to porin A protein, which inactivate the C3b component of the complement system and to the host immune system, particularly individuals with deficiency in complement C6-C9 function such as women during menses and pregnancy. Disseminated infections occur particularly in cases of asymptomatic infections.

Clinical sides include the endocervix, ascending to the uterine tubes (salpingitis, PID), anorectal areas, throat and eyes. During disseminated courses arthritis, tenosynovitis and pustules of the skin occur.

Since the growth of the organism is inhibited by metals and fatty acids, it can only be cultured on absorbing media such as agar containing heated blood. Cultivation is successful only in 50% whereas DNA detection is more sensitive (90%-95%)

Treatment: Ceftriaxone, ciprofloxacin. Resistance to penicillin is rising. Since co-infection with C. trachomatis is common, tetracyclines should be co administered.

Sampling: Culture: Swab (special transport media) to be transported to the laboratory immediately or to plate at bedside on special agar media to obtain optimal results.
DNA Probe: Swab in special transport medium

Reference Interval: Culture: Report on diagnostic finding
DNA Probe: Report on DNA detection

Neopterin, Serum

Background: Neopterin (D-erythro-6-trihydroxypropyl-pterin), synthesized by GTP-cyclohydroxylase-1, may be elevated in serum or urine during stimulation of the cellular immuno functions such as stimulation of macrophages by interferon–gamma or stimulation of monocytes derived dendritic cells.

Clinical conditions associated with increased neopterin production: Viral infections, parasites or intracellular bacterial infections, inflammatory states including rheumatoid arthritis, systemic lupus erythematodes, Morbus Crohn and other autoimmune diseases. The parameter is prognostic for malignancies and in HIV infections. It is useful in the follow up of transplant patients, in patients undergoing immunomodulatory therapy with cytokines, as well as during antibiotic or antiviral therapy. It may be helpful in differentiation of rheumatoid arthritis from osteoporosis.

Sampling: 2 mL serum

Reference Interval: 19-75 years:
0.65-2 ng/mL (2.6-8 nmol/L) or given as 95% 2.17 ng/mL (8.7 nmol/L)
Older than 75 years:
1.17-3.67 ng/mL (4.7-14.7 nmol/L) or given as 95% 4.75ng/mL (19 nmol/L)

Neurone-specific Enolase (NSE), Serum

Synonyms: Phosphopyruvate Hydratase

Background: NSE is a marker for neuroendocrine neoplasms and cerebral injury. NSE is one out of 11 enzymes of the glycolytic pathway, catalytic active to convert 2-phospho glucerate to phosphoenolpyruvate. NSE has a MW of 100 kDa composed as a dimer out of 3 subunits (alpha, beta, gamma). NSE is named the enzyme containing at least one gamma subunit.

Gamma subunit composed NSE: Synthesized in neuronal cells and neuroendocrine APUD-cells of the intestine, lung, pancreas, and thyroid gland.

Alpha-alpha subunit composed: Synthesized in glia cells and other cell types, named as a non-neuronal enolase enzyme. Beta subunit composed: synthesized in the muscle (beta-beta) and heart muscle (alpha-beta)

Small cell lung carcinoma: NSE diagnostic sensitivity 60%-93%, (CEA: 29%-69%).

Specificity against benign diseases 91%-95% (CEA: 82%-93%). In non-small cell lung carcinoma, specificity is 58%-93% (CEA 25%-68%). NSE may be the most sensitive marker in small cell lung carcinoma with 77%, followed by CYFRA21-1 with 36%; SCC 32%; CEA 28%. There is a good correlation with clinical staging, but no correlation with metastasis. NSE is a useful marker in monitoring with a predictive value of 92%.

Neuroblastoma: Values >30 µg/L of NSE are detected in 62% of children with neuroblastoma. Wilms tumor values are lower, only 20% of the patients present with >30ug/L. Good correlation with staging.

APUD cell carcinoma: Levels >12.5 ug/L are present in 11%-56% of the patients, depending on the localization of the tumor.

Seminoma: A median serum concentration of 40 µg/L in 70% of the patients has been reported.

Other malignancies: 22% of other carcinomas present levels >12.5 µg/L, usually a higher percentage in cases of metastasis.

Limitations: Increased also in benign lung diseases, in 5% of patients values > 12µg/L were reported. Increased in CNS diseases (meningitis, encephalitis, cerebral hematomas, Guillain Barré syndrome). Values > 25 µg/L occur in 2% of patients with non malignant diseases (levels >12.5 µg/L in 14% of patients). In cases of fetal brain defects up to 50% of the pregnant women presented increased NSE levels.

Sampling: 1 mL serum, strictly! Avoid hemolysis, since red blood cells contain gamma enolase causing false positive results.

Reference Interval:			
	Adults		< 12.5 µg/L
		borderline	13-25 µg/L
	Children	< 1 year	< 25 µg/L
		1-8 years	< 20 µg/L

Newborn Screen

Test Includes:
Acylcarnitine disorders
Amino acid metabolism disorders
Branched chain organic acids (leucine, isoleucine, valine) metabolism disorders
Congenital Adrenogenital Syndrome
Fatty acid oxidation disorders
Galactosemia
Glutaric aciduria type I and II
Organic acids metabolism disorders
Phenylketonuria
Thyroid function disorders

Background: General: The combined incidence of all disorders given in the test panel is about 1:4000 excluding hypothyreoses, phenylketonuria, adrenogenital syndrome, if including all parameters, the combined incidence is approx. 1:2000. Figures are evaluated in Germany.
For most of the disorders, therapies are available, but early identification is essential.
However, screening earlier than 36 hours after birth may not give valid results, screening earlier than 36 hours after birth is therefore indicated only
- if the newborn is discharged and no screening will be possible later on,
- if blood transfusion is indicated (screening has to be done before)
- if treatment with cortisone or dopamine is necessary (screen before initiation of therapy)

A re-screening has to be considered in theses cases.
For preterm or sick newborns same rules apply.
For highly preterm (less than 32 weeks of pregnancy) newborns, a second screening after corrected age of 32 weeks of pregnancy is strongly recommended.
Screening is recommended on day three of life, as standardized.

Information essentially required for the screening procedure:

- Name of the mother
- Name address of the hospital/doctor
- Date and time of specimen drawn
- Age of gestation

- Weight of the newborn
- Clear identification in case of siblings
- Parenteral nutrition of the newborn (yes or no)
- Period (hours) sample taken after birth

Sampling: is done at bed-side on a NEWBORN SCREENING TEST CARD which only can be obtained from the laboratory in advance.

The marked circles on the TEST CARD must be completely covered with the newborns blood sample. Do use native blood only (no EDTA or heparinized blood), either capillary blood or venous blood. Do not use umbilical blood. Screening has to be done before starting therapeutic use of catecholamine or corticosteroids. The blood drops are allowed to dry at room temperature, which usually takes one hour and applying heat is under no circumstances allowed for accelerating the drying!

The parameter description below serves as an overview. Not all parameters may be part of the test panel and are subject to change. New parameters may be included and may replace others. Cut off values and thresholds for pathologic values are constantly reviewed and are subject to change. The markers for diseases and cut off values are subject to change and interpretation. The laboratory provides an indication whether parameters are pathologic or borderline and may recommend a re-screening, follow ups or further, focused investigation in doubtful cases.

1. Congenital Adrenogenital Syndrome
(adrenogenital syndrome, AGS, congenitale adrenal hyperplasia)

Background: AGS an autosomal recessive disorder caused by 21 hydroxylase deficiency which is coded on chromosome 6 (6p21.3). More than 90% of AGS is caused by 21-hydroxylase deficiency. Due to altered syntheses of cortisol, more androgens are synthesized. AGS presents clinically with failure to thrive, salt-loss syndrome, atypical genitalia and a family history. The incidence in screened newborns is 1: 5 000 to 1:11 000.

Due to the lacking conversion pathway of 17-hydroxyprogesterone to 11-desoxycortisol by the enzyme 21-hydroxylase, 17 hydroxyprogesterone is accumulating to serum levels >100 µg/L (300mmol/L). Elevation of androgens such as DHEA or DHEAS are, however, not specific for AGS.

Reference Interval: Negative for AGS: Mature newborns older than 3 days:
< 60 µg/L (18.2 nmol/L)
Values are higher prior to day 3 and in preterm newborns or newborns with diseases.

2. Galactosemia

Background: Primary source of galactose is lactose, provided with milk. Three different disorders are known: galactokinase deficiency, galactose-1-phosphate uridyl transferase deficiency (GALT), and uridine-diphosphate-galactose-4 epimerase-deficiency. Overall incidence of all galactosemias are in Germany 1:55 000 and worldwide 1:18 000-1:200 000.

The majority is due to GALT.

Clinically the autosomal recessive disease present in 70 % of the cases as an acute illness at the end of the first week after birth with vomiting, icterus, rejection of feeding , hypoglycemia, ammoaciduria, and in more chronic forms with cataract (due to reduction of galactose to galactide), failure to thrive, liver enlargement, mental retardation, lethargica. Into the urine, high levels of galactose, the reduced form galactide or the oxidized form are excreted. Lactose free feeding improves within 1-3 days the situation dramatically.

Sampling: Brest feeding (milk) must have been started prior to testing. Test to be performed ideally within the first 3 days of life.

Reference Interval:

Serum: Galactose assay in serum: 0-2 mg/dL (SI: 0-1.11 mol/L)

False negative tests have been very rarely reported.

Assay for GALT: blood: 18-26 units/g of Hb

Up to 4 month after transfusions, positive test results were more frequently reported when assys are performed for GALT deficiency

Urine (not part of the test panel): Normal newborns may have physiologic elevated levels of galactose (up to 60 mg/dL) within the first days of live, in premature newborns the period may be extended to 2 weeks.

High milk intake may cause false positive levels.

3. Phenylketonuria

Background: 97% of the cases of the autosomal recessive aminoacidopathies are due to phenylalanine hydroxylase deficiency. The incidence varies by region between 1:10 000 and 1:50 000. To prevent mental retardation, early screen is necessary to initiate appropriate therapy through low phenylalanine intake below 200-300 mg per day. Hyperphenylalaninemia in mothers may result in fetal damage (microcephaly, growth retardation, heart disease), genetic counseling before pregnancy is indicated.

Sampling: Ideally, prior to testing, newborns should have a protein feed (milk).

Reference Interval:

Screening: During the first 12 hours of life >2 mg/dL (false negative 3%)
After 24 hours of life 4 mg/dL (false negative 10%-0.1%, percentage rate decreases with lifetime). If screened within the first day of life, a re-screen is indicated after 1-2 weeks.

Confirmation: >10 mg/dL suggests phenylketonuria
<10 mg/dL mild variant of phenylketonuria
Serum tyrosine, urinary pteridines and blood dihydropteridine may be tested as well.

4. Thyroxine T4 and Thyroid Stimulating Hormone (TSH)

Background: Fetal Thyroid Stimulating Hormone (TSH), T4 and T3 production starts at week 12 of gestation. Conversion of T4 to T3 is very low until week 30 of gestation, increasing to normal within the first month of life. Newborns with abnormal thyroid function show problems in mental and neurological development 4 times more often than normal newborns. Incidence of hypothyroidism is about 1:3 600.

Mature newborns: Within 24 h of life TSH increase rapidly, however values of >40 mU/L are observed in less than 1% of the newborns. TSH usually is less than 5 mU/L.

Premature newborns achieve thyroid status of adults after 4-8 weeks. A transient low level of T4 may be seen in 85% of prematures.

Sampling: Optimal collection time not earlier than 3 days after birth

Reference Interval: **T4**

Preterm newborn	
< 27 week of gestation	0.5-3.5 µg/dL
27-29 week of gestation	3.0-5.8 µg/dL
30-32 week of gestation	2.8-5.6 µg/dL
33-37 week of gestation	1.9-8.8 µg/dL

Mature newborn	Free T4	Total T4
cord blood	10-18 ng/L	60-130 µg/L
day 1 and 2	16-38 ng/L	105-260 µg/L
3-30 days	15-30 ng/L	80-200 µg/L
30-360 days	11-18 ng/L	55-140 µg/L

TSH

Preterm newborn	
19-27 week of gestation	2.6-5.5 mU/L
28-38 week of gestation	4.5-9.3 mU/L
39-42 week of gestation	2.7-5.7 mU/L

Mature newborn	
1-3 days	< 2.5-13.3 mU/L
1-4 weeks	0.6-10.0 mU/L
1-12 month	0.6-6.3 mU/L

5. Amino acid (AA) metabolism disorders

5.1 Argininemia (ARG), argininosuccinic aciduria (ASS) and citrullinemia (CIT)

Characterized by deficiency of arginase, arginosuccinate lyase (ASL) and argininosuccinate synthase (ASS) within the urea cycle. Clinically presenting with confusion, impaired speech, Reye s syndrome, mental impairment, ataxia and stupor. Laboratory abnormalities are in-

creased serum ammonia, glutamine, AST and AST and decreased urea. In argininemia, in addition orotic acid is increased.
AA markers:
For ASL and ASS: Citrate; cut off for positive >100 µmol/L (13SD)
For ARG: Arginine; cut off >132 mol/L (9SD)

5.2 Homocystinuria (HCU)
Deficiency in either cystathione-beta-synthase or deficiency in 5,10 methylenetetrahydrofolate reductase or in cobalamin or cobalamin metabolism. Clinically presents as mental or developmental retardation, thromboembolic episodes, or ectopia lentis. Defects in cobalamin metabolism results in addition in macrocytic anemia and methylmalonic aciduria.
AA marker: Methionine; cut off >67µmol/L (6SD)

5.3 Tyrosinemia (TYR)
Characterized by p-hydroxy phenyl acetic acid hydroxylase deficiency and resulting clinically in hepatic cirrhosis, renal tubular dysfunction and presenting increased plasma tyrosine. Incidence 1:155 000
AA marker: Tyrosine; cut off > 442 µmol/L (9SD)

5.4 Maple Syrup urine disease (MSUD)
A branched chain aminoacidemia characterized by a deficiency in brached chain amino acid oxidase. Clinically presenting with seizures, ketosis, and mental retardation. Laboratory results are increased urine and plasma branched chain amino acids. Incidence about 1:140 000 for Germany or 1:260 000 for the US
AA marker: Leucine; cut off >373 µmol/L.

5.5 Hypermethioninemia
AA marker: Methionine (Met) ; cut off >67 µmol/L (6SD), incidence 1:280 000. Also a marker for Homocystinuria (HCU)

5.6 Hyperornithinemia, -hyperammonemia,-hypercitrullinuria (HHH) Syndrome
AA marker: Ornithine (Orn) ; cut off >300 µmol/L (11SD)

6. Fatty acid oxidation disorders

Approximately 12 defects in fatty acid oxidation are known. Clinically presenting with hypoketotic, hypoglycemic coma induced by fasting. The most common defect is medium chain acyl CoA dehydrogenase deficiency, resulting in Reye's like syndrome with sudden infant death.

6.1 Short chain acyl CoA dehydrogenase (SCAD)
Incidence 1:33 000 and isobutyl-CoA dehydrogenase deficiency. Butyrylcarnitine (C4) or the

isomer isobutyrylcarnitine are primary markers; cut off >1.9 µmol/L (10SD)

6.2 Medium chain acyl CoA Dehydrogenase deficiency (MCAD)
Incidence 1:9 000
Marker: C8; cut off > 0.5 µmol/L (11SD)

6.3 Long chain hydroxyacyl CoA dehydrogenase deficiency (LCHAD)
Incidence 1: 220 000
Marker: C16OH (hydroxypalmitoylcarnitine); cut off > 0.1 umol/L (7SD)

6.4 Very long chain acyl CoA Dehydrogenase deficiency (VLCAD)
Incidence 1:135 000
Marker: Tetradecenoylcarnitine; cut off >0.9 µmol/L (12SD)

6.5 Carnitine palmitoyl-transferase type II deficiency (CPTII) and carnitine acylcarnitine translocase deficiency
Marker: C 16 (palmitoylcarnitine); cut off >12 µmol/L (7SD)

6.6 Glutaric aciduria type II (GA II)
Marker: C 8; cut off 0.5 µmol/L (11SD)
Further fatty acid oxidation disorders are:
multiple acyl CoA dehydrogenase deficiency, mitochondrial trifunctional protein deficiency and 2,4 dienoyl-CoA reductase deficiency

7. Organic acids metabolism disorders

7.1 Glutaric aciduria type I (GA I)
A defect in glutaryl-CoA dehydrogenase triggers clinical signs of dystonia, and encephalopathy. Laboratory tests results are metabolic acidosis, hypoglycemia, ketosis, elevated liver tests. Incidence 1:90 000.
Marker: Glutarylcarnitine (C5-DC) is the primary marker for GA I and secondary for GA II cut off: 0.21 µmol/L (8SD)

7.2 Branched chain organic acids (leucine, isoleucine, valine) metabolism disorders
Including Isovaleric academia (incidence 1:100 000), and 2-methylbutyrylCoA dehydrogenase deficiency.
Isovalerylcarnitine (C5) or its geometric Isomer, 2–methylbutyrylcarnitine is the primary marker for isovaleric academia (IVA) and 2-methylbutyrylCoA dehydrogenase (2-MBCD) deficiency.
Further organic acids metabolism disorders are:
methylmalonic acidemia, propionic acidemia and deficiencies of 3-methylglutaconyl-CoA hydratase

8. Acylcarnitine disorders

Clinically presenting with acidosis, increased anion gap, hypoglycemia, hyperammonemia.
The disorders include:
3-hydroxy-3-methylglutaryl-CoA lyase deficiency (HMG)
3-ketothiolase deficiency
3-methylcrotonyl-CoA hydratase (carboxylase) (MCC) deficiency. Incidence 1:100 000
multiple-CoA carboxylase deficiency

Marker: 3-hydroxyisovalerylcarnitine (C5OH) (cut off at 0.8 µmol/L (12SD)) or its isomers, additional markers are tiglylcarnitine (cut off at 0.08 µmol/L (6SD)) and 3-methylglutarylcarnitine (cut off at 0.12 µmol/L (SD11)).

Norepinephrine, Plasma see Catecholamines, Plasma

Norepinephrine, Urine see Catecholamines, Urine

Normetanephrine see Metanephrines, Urine

Norwalk Virus, Antigen

Synonyms: Norovirus

Background: Named after the outbreak in a school in Norwalk, Ohio in 1969, the virus classified in the family Calicivirus together with the hepatitis E virus as a small, non-enveloped, positive single stranded RNA virus. It is the most common cause of viral gastroenteritis worldwide. It is transmitted predominantly by the fecal oral route, involving often contaminated seafood and water, but person to person transmission may occur. The animal pathogenic caliciviruses are, so far, not pathogenic to humans. Due to low dose of infection, virus excretion for several weeks after recovery in the feces and a high resistance to environmental factors, the virus spread efficiently. The infection is limited to the mucosal cells of the intestinal tract, causing watery diarrheas without bleeding, vomiting, low grade fever and abdominal pain, but a high number of infections are asymptomatic. Immunity is of short duration.

Sampling: approx. 2 g of stool

Reference Interval: Report of diagnostic finding
Antigen detectable