Box A: Study Period 1 – Protocol for the evaluation for early neonatal sepsis:

The indications for early neonatal sepsis work-up in asymptomatic newborns included assessment of maternal, fetal and perinatal risk factors for infection.

a. Maternal risk factors: prolonged rupture of membranes (PROM ≥ 24 hours), premature rupture of membranes (< 37 weeks gestation), peripartum maternal fever (≥ 38°C), suspected chorioamnionitis and/or foul smelling amniotic fluids, urinary tract infection and/or Group B Streptococcus (GBS) bacteriuria, vaginal colonization with GBS.

b. Fetal-neonatal risk factors: fetal tachycardia (≥ 160 beats/minute) and/or signs of fetal distress, 5 minutes Apgar score ≤ 6, low birth-weight (≤ 2500 g), prematurity (≤ 37 weeks gestation), sepsis in twin, previous sib with GBS neonatal sepsis.

In asymptomatic newborn: One risk factor did not constitute an indication for sepsis work-up, but mandated at least 2 days of clinical follow-up. A combination of 2 risk factors mandated an evaluation for infection, which included at least complete blood count (CBC) and one blood culture.

Decision on initiation of empiric antibiotic therapy pending culture results was based on clinical condition or laboratory results (white blood cell (WBC) count ≤ 7,500 or ≥ 40,000 and/or immature / total neutrophil ratio (I/T ratio) ≥ 0.2). In borderline cases laboratory evaluation was repeated within 12 hours with C-reactive protein (CRP) (abnormal if ≥ 5 mg/L) in addition to CBC.
Routine lumbar puncture was not indicated in asymptomatic newborns\textsuperscript{22, 23}. Lumbar puncture was included in the evaluation if there were symptoms suspicious of meningitis or generalized sepsis, or at the discretion of the attending neonatologist.

In addition to these guidelines, it was accepted in our department that in every case of prolonged rupture of membranes ≥ 36 hours or maternal intrapartum fever ≥ 38.5°C to do evaluation for early sepsis in the newborn, even if the baby was asymptomatic and there were no more risk factors.

Every symptomatic newborn underwent a full sepsis evaluation (including two blood, and urine and cerebrospinal fluid (CSF) cultures) and got empiric antibiotic treatment (intravenous Ampicillin and Gentamycin) pending cultures' results. Clinical symptoms in the newborn were wide-ranged and usually non-specific.

In newborns born to mothers who were GBS carriers we followed the AAP guidelines\textsuperscript{24} with the updates made by the CDC\textsuperscript{25}. 
Box B: Study Period 2 – Main principles of the AAP protocol for the evaluation for EOS in term newborns (gestational age≥37 weeks) as they were adopted in our department (See Figures 1-3 in the AAP guidelines): 

Work-up for neonatal sepsis should be performed in the following cases:

1. **Chorioamnionitis;**

   Diagnosis of chorioamnionitis: Maternal > 38°C and at least two of the following criteria:
   
   * Maternal leukocytosis (>15000 cells/mm3)
   * Maternal tachycardia (>100 beats/minute)
   * Fetal tachycardia (>160 beats/minute)
   * Uterine tenderness
   * Foul odor of the amniotic fluid.

2. **Prolonged rupture of membranes (PROM) ≥ 18 hours;**

3. **Inadequate intra-partum antibiotic (IAP) treatment.**

   * This include every birth where there was an indication for intra-partum antibiotic (IAP) treatment to the mother (e.g. GBS carrier), but the mother got inadequate treatment (i.e. inappropriate antibiotics or treatment for less than 4 hours before delivery).

   In all these cases laboratory tests should be taken from the newborn, including: blood culture and CBC with differential. If tests are taken after 6 hours from delivery – C-reactive protein (CRP) should also be taken.

   In the case of chorioamnionitis these tests should be taken immediately after birth and wide-range empiric antibiotic treatment should be started.
In the case of PROM $\geq 18$ hours or inadequate maternal IAP the tests should be drawn after 6-12 hours, and decision regarding empiric antibiotic treatment should be based on the results.

The decision regarding initiation of empiric antibiotic treatment was very similar to the previous protocol, i.e. treatment should be initiated whenever there are clinical symptoms; white blood cell (WBC) count $\leq 7,500$ or $\geq 40,000$; and/or immature / total neutrophil ratio (I/T ratio) $\geq 0.2$.

In borderline cases laboratory evaluation could be repeated within 12 hours with (CRP) (abnormal if $\geq 5$ mg/L) in addition to CBC.

Lumbar puncture is included in the evaluation if there are symptoms suspicious of meningitis or generalized sepsis, or at the discretion of the attending neonatologist. Lumbar puncture should not be performed routinely as part of the evaluation of an asymptomatic newborn.

**Every symptomatic newborn should have a full evaluation including two blood cultures, urine and cerebrospinal fluid culture and should get antibiotic treatment awaiting culture results.**

Symptoms in the newborn are usually general and nonspecific, and are not directed to a focus of infection. Symptoms may include: hyperthermia or hypothermia, tachycardia or bradycardia, tachypnea or signs of respiratory distress, poor oxygen saturations or desaturations, apneas, cyanosis, poor peripheral perfusion, hypotension or decreased urine output, sleepiness and apathy or jitteriness and restlessness, poor appetite, vomiting and abdominal distention, jaundice, hepatomegaly, splenomegaly, or skin lesions. Clinical symptoms or signs can be either mild or severe up to full-blown multi-organ failure.