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NATIONAL STANDARD METHOD

INVESTIGATION OF GASTRIC ASPIRATES AND INFECTION SCREEN SWABS FROM NEONATES

BSOP 23

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AMENDMENT PROCEDURE

Controlled document reference	**BSOP 23**
Controlled document title	**Standard Operating Procedure for the investigation of gastric aspirates and infection screen swabs from neonates**

Each National Standard Method has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from standards@hpa.org.uk.

On issue of revised or new pages each controlled document should be updated by the copyholder in the laboratory.

Amendment Number/ Date	Issue no. Discarded	Insert Issue no.	Page	Section(s) involved	Amendment
5/ 03.05.05	4	4.1	1	**Front page**	Redesigned
			2	**Status of document**	Reworded
			4	**Amendment page**	Redesigned

STANDARD OPERATING PROCEDURE FOR THE INVESTIGATION OF GASTRIC ASPIRATES AND INFECTION SCREEN SWABS FROM NEONATES

Types of specimens: Gastric aspirates and swabs from ear canal and superficial sites

SCOPE OF DOCUMENT

This SOP describes the processing and bacteriological investigation of gastric aspirates and infection screen swabs from neonates.

INTRODUCTION

Neonatal infection refers to infection occurring during the first four weeks of life.

Infection may be superficial and localised (eg conjunctivitis, superficial skin infections) or deep. Deep infections such as pneumonia, meningitis, septic arthritis or osteomyelitis are frequently systemic, with associated bacteraemia.

The incidence of infection increases with low birth weight or prematurity and may be divided[2]:

- **early onset** - this occurs in the first 48 hours of life and is usually caused by infection ascending from the maternal genital tract or, less commonly, via the placenta[3]
- **late-onset** - this occurs after the first 48 hours of life and the organisms may be acquired from the external (eg hospital) environment. Organisms initially colonise superficial sites and the upper respiratory tract and progress to cause widespread sepsis

Infants normally become colonised within the first day of life with flora acquired from the mother and the immediate environment. Coagulase-negative staphylococci predominate on the skin and α-haemolytic streptococci in the upper respiratory tract. In the gut, *Bifidobacterium* species predominate in breast-fed infants, whereas other anaerobes and Enterobacteriaceae are found in greater numbers in bottle-fed babies.

A different pattern of colonisation occurs in infants admitted to a neonatal intensive care unit (NICU) shortly after birth. Colonisation of gut, respiratory tract and skin is delayed, often beyond the third day[4]. The use of antimicrobials and the NICU environment affects the nature of the colonising flora. There is a predominance of organisms such as Gram-negative bacilli and *Staphylococcus. aureus*.

Risk factors contributing to the development of neonatal sepsis include[5]:

- congenital abnormalities
- low birth weight
- premature birth
- prolonged rupture of membranes
- maternal fever
- protracted, difficult births
- respiratory distress syndrome
- toxaemia of pregnancy
- invasive procedures and devices (eg intravascular cannulae, shunts, prolonged artificial ventilation)
- infants of mothers who have already had one baby affected by Lancefield group B streptococcal (GBS) sepsis are at increased risk of GBS infection, as is the twin of an affected infant.

Some workers have recommended that infants at special risk of infection and colonised with pathogenic organisms should be treated with antibiotics routinely[6].

Prolonged rupture of membranes (PROM) is a predisposing factor to both fetal and neonatal infection. Organisms ascending the maternal genital tract may cause intrauterine infection (**amnionitis**). The mother may appear well despite infection of the amniotic fluid and membranes, placenta or fetus. There is increasing evidence that ascending infection is an important cause of preterm labour[7]. Attempts to prevent or control the onset of preterm labour by the increased use of antibiotics[8,9] may necessitate significant alterations to the methods used for the detection of neonatal infection, aimed at increasing both the speed and specificity of diagnosis.

A variety of microorganisms have also been isolated from the amniotic fluid, amnion and chorion, even at term and in the presence of intact membranes[10], in particular, those associated with bacterial vaginosis[11]. However, only about 8% of preterm infants whose mothers have clinical evidence of chorioamnionitis develop septicaemia. Bacteraemia, when it occurs, is often transient[12].

Unfortunately, recent randomised trials examining the use of antibiotics versus placebo in various clinical settings of preterm labour, including women with asymptomatic bacterial vaginosis, have not shown significant advantages for the neonate[13,14]. Indeed, the use of co-amoxiclav for asymptomatic women with preterm rupture of membranes was associated with a significant increase in suspected or proven necrotising enterocolitis in their infants[15]. Erythromycin had some advantages over placebo if rupture of membranes was present, but this was lost in the setting of preterm labour with intact membranes[16,15].

The clinical manifestations of neonatal sepsis, whatever the source of infection, are frequently non-specific and include respiratory distress, unstable temperature and cardiovascular depression. A bulging or tense fontanelle may be observed in meningitis.

Neonatal pneumonia of early onset may be acquired transplacentally (rubella, cytomegalovirus, listeriosis), or perinatally (associated with asphyxia or possible aspiration of the amniotic fluid)[3] or by aspiration of organisms from the birth canal. Late-onset pneumonia is usually of nosocomial origin (eg resulting from use of prolonged mechanical ventilation), although infections acquired during birth (eg *Chlamydia trachomatis*) may also present as late-onset pneumonia.

Neonatal septicaemia and meningitis are also classified into early- and late-onset and may be difficult to diagnose because of the multiplicity of associated risk factors and clinical manifestations[17,18].

Organisms associated with sepsis include[19-21, 22-26]:

- β-haemolytic streptococci, in particular Lancefield group B streptococci
- Enterobacteriaceae
- *S. aureus*
- coagulase-negative staphylococci
- *Listeria monocytogenes*
- Enterococci
- pseudomonads
- yeasts
- *Ureaplasma urealyticum*
- viruses such as echoviruses, herpes simplex

Anaerobes are an extremely rare cause of neonatal infection. Fulminant anaerobic septicaemia tends to be associated with septic abortion or post operative complications.

Lancefield group B streptococci remain the commonest cause of early-onset neonatal sepsis. There is little evidence to support transplacental infection. Organisms may be derived from the mixture of bacteria in the amniotic fluid, if present, or are acquired from the birth canal. Infection is secondary to the multiplication of organisms in the fetal lung[3].

Only a small percentage of infants colonised with these organisms develop early onset disease[27]. Those which do often have other risk factors such as PROM, low birth weight, prematurity or

congenital abnormalities. Late-onset infections associated with acquisition of the organism from the mother or healthcare personnel may also occur.

Intrapartum (ie during labour and delivery) antibiotics given to high risk mothers with Lancefield group B streptococci may prevent ascending infection and have been shown to be effective in reducing the incidence of subsequent early-onset streptococcal disease in high-prevalence populations[28-31].

Coagulase-negative staphylococci (CNS) are commonly associated with the presence of intravascular devices and are the most frequent cause of late infections in neonatal intensive care units (NICUs). They are the commonest organisms isolated from blood cultures in infants in NICUs. Although the technical difficulties inherent in collecting blood cultures aseptically from small infants means that contamination is always possible, the presence of CNS must always be interpreted in the light of the clinical status of the infant at the time blood cultures were collected and its subsequent progress. Infections with CNS may have a more insidious presentation than those caused by Gram-negative bacilli.

It is not possible to interpret the significance of CNS found in superficial swabs and gastric aspirates. The bacterial factors associated with invasion are not well understood. Genotyping studies of CNS colonising and causing infection in NICU infants, while clarifying the molecular epidemiology of the NICU CNS population, cannot predict whether colonised infants will develop infection[32].

Gram-negative bacilli are an important cause of early and late-onset neonatal sepsis.

Escherichia coli capsular type K1 is the strain most commonly associated with early onset sepsis[33-35]. In the NICU setting, it is likely that nosocomial acquisition of hospital strains of *E. coli* occurs, rather than the natural acquisition by neonates of maternal strains of *E.coli* [36].

Several outbreaks caused by multi-resistant strains of Enterobacteriaceae and pseudomonads have been reported[37-40]. Nosocomial outbreaks of meningitis[41] and enteritis[42] caused by *Campylobacter jejuni* have also been described.

L. monocytogenes is acquired through transplacental haematogenous spread from bacteraemic mothers or during passage through an infected birth canal. Neonatal listeriosis can present as early-onset or late-onset disease[43].

Listeriosis should be considered if there has been maternal febrile illness, or another clinical pointer. Amniotic fluid, placental tissues, maternal blood and vaginal secretions, neonatal blood and CSF should be cultured in addition to the routine infection screening swabs. *L. monocytogenes* DNA can be specifically detected in CSF and tissue by the polymerase chain reaction (PCR)[43]. Serology is of little use because of cross-reactivity with other Gram-positive bacteria, and culture-positive patients often have no detectable antibodies[44].

The incidence of neonatal sepsis caused by *L monocytogenes* has fallen considerably in the UK since a peak in the late 1980s due to public health education of food producers and dietary advice issued to pregnant women[45].

Other causes of late-onset sepsis include *S. aureus* and enterococci. Systemic fungal infection, particularly due to *Malassezia furfur* as a result of the use of lipid-rich total parenteral nutrition, is also increasing[46-48]. Infections with *M. furfur* associated with intravascular catheters have also been described[49]. Candida infections are also occurring more frequently[50].

INVESTIGATION OF NEONATAL SEPSIS. This is a controversial subject.

Isolation of organisms from a significant focus such as blood, CSF, skin vesicle fluid or lesion, or a properly-obtained urine (suprapubic aspirate) remains the most valid method of diagnosing systemic bacterial infection[51].

Although organisms isolated from superficial sites, gastric aspirate and amniotic fluid indicate colonization and may include pathogens responsible for disease, they do not establish the presence of active systemic infection.

Surveillance screening is performed routinely in many neonatal units and may be used to monitor trends in resistant flora and define antibiotic policies. It is seen by some to be useful for predicting the occurrence and defining the aetiology of early- and late-onset neonatal sepsis[52,53], but by others as non-contributory or misleading[54,55].

An extensive study[56] of material from the ear canal, nasopharynx, axilla, umbilicus, groin, rectum, stomach and endotracheal tube showed that isolates were rarely the same as those recovered from the blood or fluids of the cerebrospinal, joint, pleural, pericardial and peritoneal spaces. This and other studies have concluded that surface cultures are of limited value in predicting the aetiology of sepsis in the newborn[57,58]. Thus, in many units this has now been discontinued and restricted to babies who are clinically unwell.

The external ear canal is regarded as the best site to sample in the newborn with known risk factors because it may contain residual amniotic fluid. It has been shown that the ear canal is consistently culture positive in cases of early-onset GBS sepsis whereas other sites are not[59].

Gastric aspirate flora in the newborn reflects those of the amniotic fluid or birth canal[60-62]. Gram-stained smears of gastric aspirates may show the presence of polymorphonuclear leucocytes (PMNs), but these maybe maternal in origin and may not necessarily represent a fetal inflammatory response[63]. Their presence is often wrongly interpreted[3]. However, there are contradictory assertions found in the references and many authorities continue to find their presence significant[64,65].

Nasopharyngeal and endotracheal aspirates, together with an external ear canal swab in the newborn have been reported as useful indicators of early sepsis[66]. They are often sampled routinely (as described in BSOP 8) in ventilated babies on NICUs as indicators of the likelihood or actuality of late-onset infection, although the value of this procedure has been widely disputed[67,68,69]. After the first few hours of life, such specimens do not help to predict the cause of systemic sepsis[70]. However, they may be justified for the purposes of infection control surveillance.

Other diagnostic tests, not described in this SOP, which have been used to try to predict which patients will develop sepsis include:

- An immature to total neutrophil (I:T) ratio[71] (ie a left shift of the PMNs)
- C-reactive protein (CRP)[17]. The rise in CRP does not occur early enough in the process of sepsis to be a useful predictor, but persistently normal serial CRP measurements indicate sepsis is an unlikely cause of deterioration[72]
- Latex agglutination has been used for the detection of Lancefield group B streptococcal antigen in gastric aspirates[17,73] and concentrated urine[17]. Doubts have been cast on the utility of antigen detection as a diagnostic test because of both false-positive and false-negative results[74]

Localised sepsis is investigated by examination of swabs or aspirates of the particular site involved (see relevant SOP).

Eye swabs - neonatal conjunctivitis (ophthalmia neonatorum) is acquired during passage through a birth canal which is infected with *Neisseria gonorrhoeae* or *Chlamydia*. *trachomatis* and is characterised by a sticky discharge. Infection usually becomes apparent between 2-5 days (*N. gonorrhoeae*) or 5-14 days (*C. trachomatis*). Diagnosis is made by microscopy and culture of the discharge or by detection of chlamydial antigen (see BSOP 2).

About one half of all cases of neonatal *C. trachomatis* eye infection subsequently develop pneumonia[33]. This usually presents at between 4 and 11 weeks[33].

S. aureus is also a common cause of sticky eye, and in NICUs Enterobacteriaceae are not uncommon causes. *Pseudomonas aeruginosa* conjunctivitis requires aggressive treatment because devastating infection with panophthalmitis, meningitis and bacteraemia may ensue[75,76].

1.0 SAFETY CONSIDERATIONS[77-82]

1.1 SPECIMEN COLLECTION

N/A

1.2 SPECIMEN TRANSPORT AND STORAGE

1.2.1. Gastric aspirates

Sterile leakproof container in a sealed plastic bag

1.2.2. Swabs

Sealed plastic bag

1.3 SPECIMEN PROCESSING

Containment Level 2

Laboratory procedures that give rise to infectious aerosols must be conducted in a microbiological safety cabinet

Refer to current guidance on the safe handling of all organisms documented in this SOP

The above guidance should be supplemented with local COSHH and risk assessments

Compliance with postal and transport regulations is essential

2.0 SPECIMEN COLLECTION

2.1 OPTIMAL TIME OF SPECIMEN COLLECTION

Before antimicrobial therapy where possible

Gastric aspirate specimens from newborns should be collected ≤4h post delivery and before feeding

2.2 CORRECT SPECIMEN TYPE AND METHOD OF COLLECTION

Gastric aspirates should be collected according to local protocols

2.3 ADEQUATE QUANTITY AND APPROPRIATE NUMBER OF SPECIMENS

N/A

3.0 SPECIMEN TRANSPORT AND STORAGE

3.1 TIME BETWEEN SPECIMEN COLLECTION AND PROCESSING

Specimens should be transported and processed as soon as possible

3.2 SPECIAL CONSIDERATIONS TO MINIMISE DETERIORATION

Swabs should be transported in Amies transport medium with charcoal[83]

If processing is delayed, refrigeration is preferable to storage at ambient temperature. Delays of over 48h are undesirable

4.0 SPECIMEN PROCESSING

4.1 TEST SELECTION

N/A

4.2 APPEARANCE

N/A

4.3 MICROSCOPY

4.3.1. Standard

N/A

4.3.2. Supplementary

Gastric aspirates

Select a representative portion of specimen with a sterile loop and make a thin smear on a clean microscope slide for Gram staining

Swabs

Prepare a thin smear on a clean microscope slide for Gram staining

4.4 CULTURE AND INVESTIGATION

4.4.1. Pre-treatment

N/A

4.4.2. Specimen processing

Gastric aspirates

Select a representative portion of specimen with a sterile loop and inoculate a loopful on each agar plate

Swabs

Inoculate each agar plate with swab (see BSOP 54)

For the isolation of individual colonies, spread inoculum with a sterile loop

4.4.3 Culture media, conditions and organisms

For all specimens:

Clinical details/ conditions	Standard media	Incubation			Cultures read	Target organism(s)
		Temp °C	Atmos	Time		
Prolonged rupture of membranes Possible sepsis	Blood agar	35-37	5-10% CO_2	40-48h	daily	Any of the following: β-haemolytic streptococci Coagulase-negative staphylococci Enterococci Enterobacteriaceae *L. monocytogenes* Pseudomonads *S. aureus* Yeasts Pure growth of any other organism
	CLED agar	35-37	air	16-24h	≥16h	

Other organisms for consideration - *N. gonorrhoeae, C. trachomatis*, *Mycoplasma* species and *Ureaplasma urealyticum*

4.5 IDENTIFICATION

4.5.1. Minimum level in the laboratory

β-haemolytic streptococci	Lancefield group level
Coagulase-negative staphylococci	"coagulase-negative" level
Enterobacteriaceae	"coliforms" level
Enterococci	genus level
Listeria spp.	species level
Neisseria spp.	species level
Pseudomonads	"pseudomonads" level
S. aureus	species level
Yeasts	"yeasts" level

Organisms may be further identified if clinically or epidemiologically indicated

4.5.2. Referral to Reference Laboratories

Listeria species for typing

Fungi requiring identification and/or susceptibility testing

Organisms with unusual or unexpected resistance, and whenever there is a laboratory or clinical problem or anomaly that requires elucidation

4.6 ANTIBIOTIC SUSCEPTIBILITY TESTING

Refer to SOP on Susceptibility testing (BSOP 45)

REPORTING PROCEDURE

4.7 MICROSCOPY

Gram stain (if performed)

Gastric aspirates and/or ear canal swab - report on WBCs and organisms seen

4.7.1. Microscopy reporting time

Urgent microscopy results to be telephoned or sent electronically

Written report, 16 - 72h

Cases of known or suspected sepsis or amnionitis should be brought to the attention of the medical microbiologist as soon as possible

4.8 CULTURE

Report clinically significant organisms isolated **or**

Report all other growth and include an appropriate comment **or**

Report absence of growth

Also, report results of supplementary investigations

4.8.1. Culture reporting time

Clinically urgent culture results to be telephoned or sent electronically

Written report, 16 - 72h stating, if appropriate, that a further report will be issued

4.9 ANTIBIOTIC SUSCEPTIBILITY TESTING

Report susceptibilities as clinically indicated

5.0 REPORTING TO THE HPA[84] (LOCAL AND REGIONAL SERVICES AND CDSC CENTRE FOR INFECTIONS)

Refer to the following:

Individual SOPs on organism identification

Health Protection Agency publications

"Reporting to the CDR : A guide for laboratories"

"Hospital infection control : Guidance on the control of infection in hospitals"

Refer to current guidelines on CDSC and COSURV reporting

Local guidelines

Report all the following isolates and clinical conditions:

All cases of ophthalmia neonatorum, *Listeria* species and *N. gonorrhoeae*

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