

## Home Blood Collection Service

IPath Illawarra Pathology are pleased to announce that we are now able to provide a Home Blood Collection Service.

This 7-day service will be run in conjunction with Illawarra Community Health Nursing staff. These experienced and dedicated Community Health Nurses will be able to provide a home blood collection service for your patients as part of their comprehensive home nursing service.

This service will allow your patients access to our extensive pathology service with all testing being bulk billed and with no additional episode fee or out of pocket expenses being charged.

To book an appointment for your patient please contact the Community Health Centralised Referral Service on:

1300 792 755  
8.30am – 4.30pm Monday to Friday  
8.30am – 11.30am Weekends

FAX Requests to 42285623

For further information please contact:

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4222 5362

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0411446169

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Please contact our Customer Liaison Officer to obtain your personalised IPath Illawarra Pathology request form, in order that you can fax through your requests to the Community Health Centralised Referral Service.

## Diagnosis of SARS

SARS or Severe Acute Respiratory Syndrome is a recently recognised contagious form of pneumonia. It was first identified in southern China in late 2002 and has now been detected in at least 29 countries. The syndrome appears to be caused by a previously unknown coronavirus, currently called SARS coronavirus (SARS Co-V). Other coronaviruses are known to cause upper respiratory tract infections such as the common cold in humans and rarely cause lower respiratory tract infections in elderly or compromised individuals.

## Clinical Features

The most common symptoms of SARS include:- fevers (100% cases); chills, rigors or both (28-73%); nonproductive cough (57-69%); myalgias (49-60%); headache (35-56%); and dyspnoea (~42%).

Less common symptoms include:- sputum production (5-29%); sore throat (13-23%); coryza (2-23%); nausea and/or vomiting (~19%); and diarrhoea (20-23%).

Physical examination on presentation may reveal tachycardia (~46%), tachypnoea (~37%) and inspiratory crackles on chest auscultation (~26%).

Chest x-ray (CXR) on presentation has been reported to be abnormal in ~75% of cases. Both unilateral and bilateral areas of air-space opacity can be seen. Most patients with a normal CXR on presentation will develop air-space opacities during the course of the illness or have ill-defined peripheral ground glass opacities on thoracic CT scans.

Suspicious haematology and clinical chemistry laboratory features include:- lymphopaenia (~70%); mild thrombocytopenia (up to 45% of cases); elevated lactate dehydrogenase levels (70-80%); hypocalcaemia (~60%); and elevated creatine kinase levels (32-56%).

## Case Definitions

For the purposes of surveillance, infection control and public health measures, the World Health Organisation (WHO) has formulated case definitions for SARS:

## Suspect Case

- 1) A person presenting after 1/11/2002 with a history of:
  - High fever (>38°C)
  - AND
  - Cough or breathing difficulty
  - AND one or more of the following exposures during the 10 days prior to onset of symptoms

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- Close contact with a suspect or probable case of SARS
- History of travel to an area with recent local transmission of SARS
- Residing in an area with recent local transmission of SARS.

2) A person with an unexplained acute respiratory illness resulting in death after 1/11/2002, but on whom no autopsy has been performed AND one or more of the exposure histories listed in (1) above.

#### Probable Case

- 1) A suspect case with radiographic evidence of infiltrates consistent with pneumonia or respiratory distress syndrome (RDS) on CXR.
- 2) A suspect case that is positive for SARS coronavirus by one or more laboratory assays.
- 3) A suspect case with autopsy findings consistent with the pathology of RDS without an identifiable cause.

Close contact is defined as having cared for, lived with, or had direct contact with respiratory secretions or body fluids of a suspect or probable case of SARS. As of 21/5/2003, areas with recent local transmission of SARS include:

- China (Beijing, Guangdong, Inner Mongolia, Shanxi, Tianjin, Hebei, Hubei, Jilin, Jiangsu, Shaanxi)
- Taiwan (Taipei)
- Hong Kong
- Singapore

#### **Laboratory Diagnosis**

The clinical presentation, CXR appearances, clinical chemistry and haematology laboratory features are not specific for SARS. Such findings can occur in other forms of pneumonia caused by "atypical" pathogens, extracellular bacteria such as *Streptococcus pneumoniae*, or other respiratory viruses. Laboratory tests for SARS-CoV (PCR and virus isolation) have recently become available in the two NSW public health reference laboratories (Centre for Infectious Diseases and Microbiology Services, ICPMR, Westmead and South Eastern Area Laboratory Services, Prince of Wales Hospital). The sensitivity of these assays is unknown but appears to depend on the type of specimen (nasopharyngeal aspirate > nasopharyngeal swab > throat swab > faeces) and the timing of specimen collection after symptom onset (early > later). Sensitivity can be improved if multiple specimens and multiple body sites are tested.

Serological tests for SARS-CoV specific antibodies appear to be more sensitive than PCR or virus isolation, particularly in the later stages of the illness. However it can take 21 days after symptom onset for SARS-CoV antibodies to become detectable. As at 21/5/2003 serological tests for SARS-CoV are not available in NSW but it is anticipated that such tests will be available in the near future.

As a result of these diagnostic limitations, SARS remains a diagnosis of exclusion. Extensive laboratory evaluation for other known causes of pneumonia is important since identification of an alternative diagnosis to explain the

patient's illness allows SARS to be excluded. This then allows the lifting of quarantine and the stringent infection control measures that are applied to suspect and probable SARS cases to prevent further transmission.

#### **Tests Currently Recommended in the Investigation of Suspected or Probable SARS**

1. **Blood:**
  - a) Serology
    - Acute sample (two 10 ml serum gel tubes) with a convalescent sample collected 10-14 days later.
    - Serology tests requested: Chlamydia sp., *Mycoplasma pneumoniae*, Legionella sp., Q fever, Influenza A and B, Adenovirus, RSV, Parainfluenza and storage for SARS-CoV antibody testing (when it becomes available)
    - If no alternative diagnosis is made, then request a further convalescent sample, collected at least 22 days after symptom onset, for storage and subsequent SARS-CoV antibody testing (when it becomes available).
  - b) Blood cultures (request prolonged incubation)
  - c) 5-10ml whole blood in an EDTA tube for SARS-CoV PCR testing.
2. **Respiratory tract samples:**
  - a) Nasopharyngeal aspirate (NPA), if possible and using appropriate infection control precautions during collection, or, if not possible (patients quarantined in the home), then a throat swab and nasopharyngeal swab collected into viral transport medium (available from the Microbiology Department) for respiratory virus and SARS-CoV culture, respiratory virus immunofluorescence antigen detection and PCR (includes Influenza A and B, RSV, Adenovirus, Parainfluenza), SARS-CoV PCR and Metapneumovirus PCR.
  - b) A dry throat swab (using a Pertussis pernasal swab without transport media, available from the Microbiology Dept) for *Chlamydia pneumoniae* PCR and *Mycoplasma pneumoniae* PCR
  - c) Expecterated sputum (if cough is productive) – request Gram stain, routine culture and Legionella culture.
3. **Urine** for Legionella urinary antigen
4. **Stool** for SARS-CoV PCR and culture

Tests for other pathogens such as *Bordetella pertussis*, *Mycobacterium tuberculosis*, *Pneumocystis carinii*, leptospirosis, melioidosis, CMV, EBV, or even malaria may also be required depending on the clinical features. For further information please contact the Medical Microbiologist on duty.

All cases of suspected or probable SARS should be notified immediately to the Public Health Unit. The Medical Microbiologist on duty should also be notified (contact via Wollongong Hospital switchboard). All pathology specimens must be enclosed in leak-proof containers with secure closures. Each specimen container should be transported to the Pathology laboratory in its

own dedicated biohazard specimen bag, not bundled together with other specimens. Specimens should be immediately hand-delivered to the Pathology laboratory. The laboratory staff should be notified by telephone in advance of the specimen arriving in the laboratory so that appropriate infection control precautions can be taken. The pneumatic tube delivery system should not be used to transport suspected or probable SARS specimens. The request form accompanying all such specimens should be clearly labeled as "Suspect or Probable SARS".

SARS is a rapidly emerging contagious pneumonia and new information about the syndrome and the responsible pathogen is accumulating each day. The above recommendations may change as more data becomes available. Updated diagnostic information will be posted on the IPath Illawarra Pathology intranet site. For information regarding transmission of SARS-CoV and infection control measures please refer to the Illawarra Health Intranet or the NSW Health Internet site

<http://www.health.nsw.gov.au/public-health/alerts/sars/index.html>.

References available on request.

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### Diagnosis of Subarachnoid Haemorrhage by Detecting Xanthochromia in Cerebrospinal Fluid

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#### INTRODUCTION

About 1% of patients presenting to the Emergency Department have headache, often of acute onset, as their chief complaint (6). One study has reported that subarachnoid haemorrhage (SAH) is the cause in about 25% of these cases (6). The term, SAH, describes the spontaneous arterial bleeding into the subarachnoid space, usually from a cerebral aneurysm (4).

When available immediately, computerized tomography scanning (CT) is the investigation of choice in cases of suspected SAH (5). CT provides a high initial sensitivity (Table 1) for the detection of blood in the basal cisterns of the brain (4). The test is also noninvasive and provides valuable information about the origin and extent of haemorrhage and the presence of complications (9).

**Table 1. Approximate probability of recognizing an aneurysmal haemorrhage after the initial event (6)**

	CT	Spectrophotometry
Day 0	95%	100% <sup>1</sup>
Day 3	74%	100%
Day 7	50%	100%
Day 14	30%	100%
Day 21	~0%	70%
Day 28	0%	40%

<sup>1</sup> If done after 12 hrs

From Table 1, it is obvious that about 5% of cases will have a normal CT scan if the test is done within 24 hrs of the haemorrhage (3). This figure increases the longer the delay. In

such cases a lumbar puncture can be done to include or exclude the diagnosis of SAH (3-6). The value of a lumbar puncture lies in its high sensitivity for detecting blood following SAH the ability to stratify patients into low and high-risk groups (7). Patients who are positive or borderline positive for xanthochromia a cerebral angiogram may be done to confirm the presence or absence of an aneurysm (2).

### MECHANISM FOR THE DEVELOPMENT OF XANTHOCHROMIA IN CSF

RBC are present in the CSF of virtually all cases of SAH. They gradually lyse and disappear over a variable period of time from 6 to 30 days (6, 9). Lysis of RBC following an aneurysm results in haemoglobin being released into the CSF. Although the average survival of RBC in blood is 120 days, lysis of RBC occurs within 2 to 4 hrs in CSF (4). The presence of haemoglobin in CSF stimulates an increase in the activity of the Heme Oxygenase (HO) enzyme system located in the arachnoid and choroid plexus of the brain. Over a period of about 12 hrs enzyme activity increases, catalysing the conversion of heme derived from lysed RBC to bilirubin (8). These two pigments, haemoglobin and bilirubin, are responsible for xanthochromia in CSF (6).

Although haemoglobin can be detected as early as 2 hrs after entry of RBC into CSF, xanthochromia is not reliably present until after 12 hrs (6). For this reason a lumbar puncture is best delayed until 12 hrs after the onset of symptoms (3, 6).

### METHOD FOR THE DETECTION OF XANTHOCHROMIA IN CSF

RBC are present in the CSF of virtually all cases of SAH. During the lumbar tap, the CSF sample should be observed for blood staining. If present, serial samples should be collected to aid in the differentiation between SAH and a traumatic tap. The CSF should be centrifuged within 6 hrs and the supernatant inspected. If the CSF sample is xanthochromic to the naked eye then no further investigation is necessary.

Investigations have confirmed that naked eye investigations of CSF is not sensitive enough to detect small quantities of pigment after a minor bleed (5, 6). An objective method for detection of xanthochromia, if the sample is clear to the naked eye, is spectrophotometry (5). Centrifuged CSF is scanned within the range of 300 to 600 nm. In the absence of other complicating factors (see below) peaks at 415 nm for oxyhaemoglobin and/or 450 for bilirubin confirm the presence xanthochromia (3, 10). It is rare to detect bilirubin alone because oxyhaemoglobin is usually detected for at least 3 weeks after SAH (4). If bilirubin alone is detected, quantitation is required with a correction for non-haemorrhagic sources, such as the influx of protein-bound bilirubin across the blood-brain barrier (4).

### PROBLEMS WITH DETECTING XANTHOCHROMIA IN CSF

#### 1. False positive result due to blood-staining from a traumatic tap

Since RBC are almost universally present in CSF taken soon after onset of symptoms, there is no consensus on what number of RBC constitutes a traumatic tap (9). However, confusing a true SAH with a traumatic tap can have significant morbidity and mortality consequences. Conversely, incorrectly concluding that a traumatic tap is a true SAH may expose the patient to potentially risky procedures such as angiography (9).

The likelihood of a false positive is related to the degree of bloody contamination and to the delay in centrifugation of CSF (4). One study showed that CSF samples that are centrifuged within 15 min of collection and have a cell counts of <40,000/mL are unlikely to produce false positive results (4). Another study, using a cutoff of 1000 RBC/ml yielded a traumatic tap frequency of 10.1% (9).

## 2. False positive result due to age-deterioration of CSF

The CSF sample should be fresh. One study has shown that if CSF containing  $10^9$  RBC/L is allowed to stand at room temperature for more than 12 hrs, sufficient *in-vitro* haemolysis will occur to cause a discernible haemoglobin peak (1).

## 3. False positive result due to elevated CSF protein concentration

CSF protein concentrations greater than 1 g/L result in an extinction at 415 nm greater than 0.023, without there necessarily being a demonstrable oxyhaemoglobin peak (1). Therefore, an actual peak must be present.

## 4. False positive result due to serum contamination of CSF

If sufficient serum enters the CSF from a traumatic tap (usually in excess of 100,000 RBC/mL) the CSF may appear xanthochromic (9).

## 5. False Positive results from other causes

### Table 2. Non-aneurysmal causes of xanthochromia in CSF (4, 6, 9).

- Serum Bilirubin >171 umol/L
- CSF Protein >1 to 1.5 g/L because of the albumin-bound bilirubin
- Dietary hypercarotenaemia
- Malignant melanomatosis
- Oral intake of Rifampin

## 6. False negatives result due to early investigation

The time frame required for blood from the brain to reach the lumbar region is not known (7). Therefore, false negative rates of about 2% and 7% have been reported when CT is performed within 12 hrs and 24 hrs respectively, after the onset of headache. Lumbar puncture done within 12 hrs can also give false negative results (4).

## DIFFERENTIATING BETWEEN A TRAUMATIC TAP AND TRUE XANTHOCHROMIA

Xanthochromia due to haemoglobin may be detected in the absence of previous SAH if the traumatic taps results in heavy bloodstaining and *in vitro* haemolysis (1, 4, 5). As many as 20% of lumbar punctures may result in a traumatic tap. This reduces the specificity of spectrophotometry and effectively complicates the diagnosis of SAH (5, 7, 9). Two practical methods have been proposed to help make the distinction between a traumatic tap and SAH (4-6):

- a) After a traumatic tap, sequential CSF samples will appear less blood-stained. The RBC count will fall with each CSF sample collected during the tap. By contrast, in SAH, the RBC count should remain constant. However, this criterion does not exclude the co-existence of traumatic tap and SAH.
- b) A traumatic tap does result in the formation of bilirubin. Several studies have demonstrated that incubation of RBC in CSF for extended periods (>3 days) cause an increase in haemoglobin but fail to produce an increase in bilirubin (3, 8). This is because the formation of bilirubin requires the enzyme Haemoglobin Oxidase present in the brain. Conversion of haemoglobin to bilirubin does not occur *in vitro*. Therefore, the presence of bilirubin in CSF supports the diagnosis of SAH. However, its absence does not exclude the existence of SAH if CSF within 12 hrs after onset of symptoms.

## ASSAY PERFORMANCE

Because of the critical importance of this test for positive patient outcomes IPath Illawarra Pathology Service performs this assay on a urgent basis 24 hrs per day, 7 days per week. A minimum of 0.5 mL CSF sample is required for this assay. Unlike samples that are only for culture, CSF samples for xanthochromia must be protected from light. This requirement is to prevent loss of the photo-sensitive bilirubin.

## REFERENCES

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## Blood Transfusion Improvement Collaborative (BTIC)

The Blood Transfusion Department of IPath Illawarra Pathology are involved as part of the Illawarra Health BTIC group. This BTIC Group is part of a state wide improvement project being undertaken by a consortium of NSW experts in blood transfusion and quality improvement methods.

What are the aims of BTIC?

To improve the appropriate use of red cells in accordance with the recently published NHMRC/ANZSBT Guidelines

To reduce inappropriate use of red cells in elective transfusions in clinically stable patients by 50% in 12 months.

To promote the spread of successful transfusion practice improvement strategies throughout all hospitals in NSW.  
To develop performance measures that can be used to track appropriateness of blood transfusion practice.

For a copy of the Guidelines please click on to the [IPath Illawarra Pathology website](#)