NATIONAL STANDARD METHODS: ASSURING QUALITY IN UK Portugal

Valerie Bevan
Department for Evaluations and Standards Laboratory Centre for Infections Health Protection Agency

October 2008
Structure of talk

Background to:

• Health Protection Agency (HPA)
  • Centre for Infections (CfI)
  • Department for Evaluations, Standards and Training (DEST)

• Standardising methods
  • How we develop them
  • Why
  • What they look like
  • How you can access them
  • Department for Evaluations, Standards and Training (DEST)
Main purpose of HPA

Dedicated to protecting people’s health

http://www.hpa.org.uk
Centre for Infection

Reference Microbiology

Epidemiology / surveillance
Quality Assurance

Patient

Test selection
Patient preparation
Specimen collection
Specimen preservation

Specimen reception
Unique lab identifier
Analysis
Analytical validation
Clinical validation

Results dispatched
Results interpreted
Clinical management

Accreditation checks compliance

Lorian cycle (1982)
What do we mean by assuring quality?

- Working to a good quality system
- Adequate quality assurance (EQA, IQC, IQA)
- Having a ‘service culture’ focussed on the user / clinician and patient
- Good reporting of all diseases for surveillance, according to local requirements
- Timeliness of reporting
- High quality clinical advice and interpretation of results
- Public health focus
- Meeting accreditation standards
- Having good standard operating procedures
- Quality manual
Department for Evaluations, Standards and Training (DEST)

Head of Department & Laboratory Director:
Valerie Bevan

Standards Unit

Quality System Unit

Evaluations Unit (MiDAS)

Quality Control Reagents Unit

Education & Training Unit

Ruhi Siddiqui
Professor Ian Sharp
Dr Keith Perry
Joe Vincini
Dr Satnam Sagoo
National Standard Methods (NSMs)

Comprehensive collection of > 200 well referenced:

- clinical microbiology standard operating procedures
- algorithms (virology and serology)
- guidance notes
Over 200 National Standard Methods

• 84 Clinical bacteriology
  BSOPs 37
  BSOP IDs / BSOP TPs 47
• 41 Virology / serology algorithms and SOPs
• 40 Food, water and environmental
• 39 Guidance Notes
• 5 Syndromic algorithms (in development)
INVESTIGATION OF FAECES SPECIMENS FOR BACTERIAL PATHOGENS

BSOP 30
Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections

INVESTIGATION OF GASTROINTESTINAL TRACT AND ASSOCIATED SPECIMENS FOR BACTERIAL PATHOGENS

BSOP 11
Issued by Standards Unit, Evaluations and Standards Laboratory
Centre for Infections
SOPs (NSMs)

Index
Amendment procedure
Scope
Introduction
Technical information
Safety considerations
Specimen collection
Specimen transport & storage
Specimen processing
Reporting
References
Working Group Meetings

• All day meetings 2-4 times a year for each group

• Working Groups:
  
  Bacteriology
  Virology
  Syndromic Algorithms
  Molecular Technology (being formed)
  Food, Water and Dairy

• Multi-professional composition:
  
  Chairperson
  Members of the Standards Unit - Unit Head
  - Co-ordinator/Secretariat
  Medical microbiologists
  Healthcare scientists
  Stakeholder representatives
  Editor (does not attend meetings)
Stakeholders – experts and stakeholders
Process

1. Draft
2. Write
3. SM Working Group
4. Standards Unit
5. Consult
6. 1st draft Via Web
7. Consult
8. 2nd draft Via Web

Health Protection Agency
Access

- Final versions in Adobe .pdf format
- Access to drafts and reviews (in MS Word) requires password
- Over 1300 passwords in 75 countries to access our website for NSMs

www.hpa-standardmethods.org.uk
www.evaluations-standards.org.uk
www.hpa.org.uk
Welcome to the Standards Unit

Who are we?

The Standards Unit develops National Standard Methods (including SOPs, Algorithms and Guidance Notes) for clinical and public health microbiology. We do this with colleagues from throughout the UK in multiprofessional working groups and in conjunction with professional organisations.

We also communicate possible Technical Adverse Incidents to participating laboratories in conjunction with the Medicines and Healthcare Products Regulatory Agency and relevant manufacturers and suppliers.

Click here for more information.

Where do we fit in?

Standards Unit is part of the Evaluations and Standards Laboratory in the HPA’s Centre for Infections. The Standards Unit is one of 4 units in the Evaluations and Standards Laboratory.

The other three Units undertake the following:

- MiDAS assesses the performance of microbiological in vitro diagnostic devices (IVDDs) and associated equipment used to diagnose and manage infection
- The Quality Control Reagents Unit produces Quality Control Reagents for virology / serology
- The Quality Systems Unit provides support to all Laboratories within the Centre for Infections, Colindale on quality related matters
National Standard Methods (VSOP)

Laboratory testing strategies

Minimum acceptable standards

Frontline testing

Confirmatory strategies

- algorithm flowchart format

or

- working standard operating procedure (SOP) format

Some with limited access eg H5N1 influenza
Anti HIV screening VSOP 11

Anti-HIV 1 and 2 Screening Test
A “third generation” assay based predominantly on recombinant antigens OR
A “fourth generation” combined anti-HIV/p24 Ag assay

UNREACTIVE SPECIMEN

HIV-related signs and symptoms present. Define recent exposure.
Request repeat sample in 7-10 days. Serum for antibody & antigen testing. EDTA blood for PCR.

No HIV-related signs and symptoms
No definite recent exposure

REPORT:
NO EVIDENCE OF HIV INFECTION
Follow-up periodically if “AT RISK”

REACTIVE SPECIMEN

Re-test in screening assay or a second assay of at least equivalent sensitivity from clot or original tube

REACTIVE

REFER LOCALLY REACTIVE SAMPLE TO CONFIRMATORY LAB
Collect a follow-up specimen as soon as convenient

UNREACTIVE

POSSIBLE TECHNICAL PROBLEM
Reassess assay equipment and procedures

DIAGNOSIS OF HIV NOT TO BE REPORTED UNLESS:

1. A confirmatory laboratory has issued a report confirming tests on the first specimen are consistent with HIV infection, AND

2. The follow-up specimen has been shown to have equivalent or greater reactivity than the first in the assay in use by the screening laboratory.
Hybridisation Probes

Primers (if not using Health Protection Agency QCMV LC Kit)
Prepare 1 μm stock of the forward primer and 4.5 μm stock of the reverse primer and store at -20°C or 4°C. The reverse primer is labelled with LC Red 640 at the 5' end and should be protected from light. Primer nucleotide sequences are given in Appendix 1.

Probe (if not using Health Protection Agency QCMV LC Kit)
Probe should be labelled with fluorescein at the 3' end. Prepare 1μm stock and store protected from light at -20°C or 4°C. The probe nucleotide sequence is given in Appendix 1.

Plasmodium derived control material - quantitative standards
Supplied with Health Protection Agency QCMV LC Kit or made in-house. If made in-house these should be diluted in 10ng/μl hepanin sperm DNA and stored at -20°C or 4°C.

5.3 PCR Amplification

5.3.1 Health Protection Agency QCMV LC kit

If using the Health Protection Agency QCMV LC Kit prepare reaction mix as follows. The volumes shown are sufficient for two reactions. Prepare a batch of reaction mix sufficient for the number of samples, quantitative standards and controls to be tested.

<table>
<thead>
<tr>
<th>Component</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>FastStart DNA Master hybridization probe</td>
<td>2.0 μl</td>
</tr>
<tr>
<td>DNase-free MgCl₂</td>
<td>0.6 μl</td>
</tr>
<tr>
<td>MgCl₂ (supplied with Mastermix)</td>
<td>3.2 μl</td>
</tr>
<tr>
<td>Water (supplied with Mastermix)</td>
<td>3.8 μl</td>
</tr>
</tbody>
</table>

Dispense 15 μl of the reaction mix straight into the LightCycler capillary. Add 5 μl of the DNA extract, quantitative standard or control.

Cap the capillaries and spin at 1000 rpm for 10 seconds to deposit the reaction mix at the base of the reaction capillary. Transfer the capillaries to the Roche LightCycler.

Designate quantitative standards as ‘standard’ and enter the values as:

- QSA = 5.600E+4 (5 x 10⁴)
- QSB = 5.600E+4 (5 x 10⁴)
- QSC = 5.600E+3 (5 x 10³)
- QSD = 5.600E+2 (5 x 10²)
- QSE = 5.600E+1 (5 x 10¹)

Amplify DNA using the following parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protocol</td>
<td>65</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>600</td>
</tr>
<tr>
<td>Time at Temperature (sec)</td>
<td>20</td>
</tr>
<tr>
<td>Transition rate (°C/sec)</td>
<td>None</td>
</tr>
<tr>
<td>Fluorescence acquisition</td>
<td>None</td>
</tr>
</tbody>
</table>

INVESTIGATION OF CYTOMEGALOVAIRUS INFECTION BY ROCHE LIGHTCYCLER™ PCR

Issue 1.1 Issue date: 03.05.05 Issued by: Standards Unit, Evaluations and Standards Laboratory on behalf of the
Virology Working Group on Standard Operating Procedures and Quality Page 7 of 14
Reference no: VSCP 561.1
This SOP should be used in conjunction with the series of SOPs from the Health Protection Agency.

Email: standards@hpa.org.uk

www.evaluations-standards.org.uk
www.virology-standards.org.uk

Investigation of Cytomegalovirus Infection by Roche LightCycler™ PCR

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4.6 Epstein-Barr virus

Encephalitis and meningitis are recognised as uncommon complications of glandular fever. Acute EBV accounts for 4.5% of infections related to neurological disease, while reactivated EBV is reported to be an important cause of neurological complications in paediatric patients.

4.6.1 Diagnosis of EBV encephalitis

Detection of EBV DNA PCR on CSF is diagnostic for CNS disease (provided there has not been a traumatic tap). When quantitative PCR is done, levels are relatively high in both EBV CNS lymphoma and acute EBV encephalitis cases, and lower in post-infectious EBV-related syndromes such as Guillain-Barré syndrome, acute demyelinating encephalitis and transverse myelitis. Antibody studies on serum may support a diagnosis of acute EBV encephalitis for example, when EBV VCA IgM is positive and anti-EBB negative.

4.7 Herpes simplex virus

Herpes simplex virus infects the CNS under several circumstances. In the rare congenital herpes simplex infection CNS damage results from intrathecal herpes encephalitis. This can occur at any gestational age. In the neonatal period HSV acquired from the mother perinatally may present as localised vesicles, encephalitis with or without skin or mucosal involvement, and as disseminated HSV involving the CNS and other organs.

The mortality rate for untreated neonatal CNS disease is 50 to 80%. Survivors of neonatal herpes encephalitis treated with acyclovir or vidarabine are more likely to suffer neurological sequelae with HSV type 2 infection than with HSV type 1. MRI scanning frequently shows haemorrhagic lesions in the cerebral cortex in the infected neonate.

In sexually active individuals, the primary attack of genital HSV type 2 infection is not infrequently associated with evidence of meningitis, particularly in women. Genital lesions are however present only in a minority of cases of HSV type 2 meningitis. Although herpes simplex meningitis is usually self-limiting with spontaneous recovery, severe cases can occur particularly in patients who are immunocompromised.

Herpes simplex encephalitis is the most common cause of sporadic encephalitis affecting 1 to 4 per million population per year. Over 95% of cases are due to HSV type 1, including both primary HSV infection and reactivation of latent HSV. The disease presents with a fulminant illness in the two weeks preceding the onset of neurological symptoms. Lesions are characteristically in the temporal lobes. Early administration of acyclovir is crucial in improving outcomes.

4.7.1 Diagnosis of herpes simplex meningitis and encephalitis

A combination of detection of intrathecal production of antibody and PCR on CSF is the most reliable diagnostic strategy. In HSV encephalitis a second CSF sample for PCR is advised 14 days after treatment. If the HSV PCR is positive, retreatment is recommended. It should be noted that after the first few days after the onset of HSV encephalitis CSF PCR may be negative in a significant number of patients (over 50%). Quantitative PCR may have a role in assessing progress and monitoring response to therapy.

Introduction of HSV PCR tests resulted in many atypical or mild cases being recognised, particularly with type 2.

In neonatal herpes encephalitis CSF HSV PCR has high sensitivity. Further sensitivity can be gained by testing blood as well as CSF by PCR. HSV DNA detected from neonatal dried blood spots may allow early diagnosis where symptoms are vague. Virus isolation or PCR from other sites such as skin vesicles, are also relevant.

4.8 Cercopithecine herpesvirus

The cercopithecine herpesvirus 1 (monkey B virus) is endemic among macaque monkeys, in which it causes mild localised lesions and remains latent. Some 70% of captive adult
**Syndromic algorithms**

**Hepatitis in Neonates**

- **Clinical history / Patient group**
- **Sample type**
  - Serum / Blood
  - Serum or EDTA / Urine
- **Initial testing**
  - HAV: anti-HAV IgM (VSOP 27)
  - HBV: HBsAg, anti-HBC IgM (VSOP 4)
  - CMV (Quantification) (VSOP 35)
- **Supplementary testing**
  - Blood cultures (BSOF 37)
  - HEV: anti-HEV IgM (VSOP 6)
  - Adenovirus
  - EBV (VSOP 26)
  - HHHSV 6 (VSOP 23)
  - Aspergillosis
  - Candida

**Immuno-compromised**

- **Glandular fever type symptoms (QSOP 44)**
  - EBV: EBV specific serology or heterophile antibody test (VSOP 26)
  - CMV: CMV IgM (VSOP 28)
  - HIV: HIV antibody (VSOP 14)
  - T. pallidum (VSOP 44)
  - Toxoplasma gondii
  - HHHSV 6 (VSOP 23)

**Isolation**

- PCR
- Serology
- Isolation
Syndromic Algorithm

Acute Infective Hepatitis

1. Clinical history / Patient group
   - No additional history
   - IDU with acute Hepatitis
   - Foreign travel
   - Fulminant
   - In chronic HBV carrier (HBSAg positive)
   - Immune-compromised
   - Glandular fever type symptoms (QSP 44)
   - Occupational / Farming Water exposure
   - Suspected bacterial sepsis

2. Sample type
   - Serum
   - Serum / Blood
   - Serum or EDTA / Urine
   - Serum
   - Serum / Blood
   - Blood

3. Initial testing
   - HAV: anti-HAV IgM (QSP 27)
   - HBV: HBSAg (QSP 4)
   - HBV: anti-HAV IgM (QSP 27)
   - HCV: anti-HCV (to assess pre-existing HCV) (QSP 5)
   - HAV: anti-HAV IgM (QSP 27)
   - HBSAg (QSP 4)
   - HAV: anti-HAV IgM (QSP 4)
   - HBV: anti-HAV IgM (QSP 27)
   - HBSAg (QSP 4)
   - HAV: anti-HAV IgM (QSP 4)
   - HBC IgM (QSP 4)
   - TCV: HCV rt PCR (QSP 27)
   - EBV: EBV specific serology or heterophile antibody test (QSP 36)
   - Leptospira: Leptospira IgM (QSP 37)
   - Blood cultures (QSP 37)
   - Blood cultures (QSP 37)
   - Blood cultures (QSP 37)
   - Blood cultures (QSP 37)
   - Blood cultures (QSP 37)
   - Blood cultures (QSP 37)

4. Supplementary testing
   - Consider other pathogen depending upon patient age and disease (QSP 27)
   - HAV: anti-HAV IgM (QSP 27)
   - HBV: HBSAg (QSP 4)
   - HCV: anti-HCV (to assess pre-existing HCV) (QSP 5)
   - HAV: anti-HAV IgM (QSP 27)
   - HBSAg (QSP 4)
   - HCV: anti-HCV (QSP 5)
   - HEV: anti-HEV IgM (QSP 6)
   - Brucella
   - Consider other foreign Yerines
   - HEV: anti-HEV IgM (QSP 6)
   - HCV: anti-HCV (QSP 5)
   - HEV IgM (QSP 5)
   - HCV rt PCR (QSP 5)
   - CMV: CMV IgM (QSP 28)
   - HBV: anti-HAV IgM (QSP 27)
   - HBSAg (QSP 4)
   - Adenovirus
   - EBV (QSP 26)
   - HHV-6 (QSP 23)
   - Aspergillosis
   - Candida
   - CMV: CMV IgM (QSP 28)
   - HBSAg (QSP 4)
   - Adenovirus
   - EBV (QSP 26)
   - HHV-6 (QSP 23)
   - Toxoplasma gondii
   - HAV: anti-HAV IgM (QSP 27)
   - HBV: HBSAg (QSP 4)
   - CMV: CMV IgM (QSP 28)

Legend:
- PCR
- Serology
- Isolation
Syndromic algorithms

Bench level guide

- ‘Front end’
- Link bacteriology and virology testing
- Make informed decisions
- Selection of tests
- Appropriate samples
- Appropriate reporting
- Cross referencing to relevant NSMs
Why use National Standard Methods?

- Assist in Accreditation, Quality, Safety, Staff training
- Links with regulatory bodies, professional microbiology organisations, public health and epidemiologists
- Facilitates the movement of staff across laboratories
- ‘Honest broker’ when laboratories merge
- Well referenced, provide advice, existing best practice
- Improve the quality of epidemiological data
- Removes the need for labs to produce methods from 1st principles
- Wide consultation (>1300 password holders), regular review periods ensure documents are current
- Enables rapid responses to changes required
- ‘Accessible to all for FREE via the Internet (and with a password)
Feedback received

“The introductions are very useful especially for the BMS examinations”

“You provide reassurance to laboratories regarding methods employed”

“The methods provide a good basis when applying for funding for infrastructure”
What we are moving towards…

Documents to be interactive
Documents taking presenting symptom to the diagnosis
Facilitating molecular diagnostics
Improving links with clinical microbiology
Book of introductions
‘Pathopedia’ (with Royal College of Pathologists)
Department for Evaluations, Standards and Training (DEST)

Head of Department & Laboratory Director:
Valerie Bevan

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Education & Training Unit

Ruhi Siddiqui

Professor Ian Sharp

Dr Keith Perry

Joe Vincini

Dr Satnam Sagoo
Report 06054

Four nucleic acid amplification tests for *Chlamydia trachomatis* in urine specimens

October 2007

See websites for published reports

www.pasa.nhs.uk/cep

www.hpa-midas.org.uk
Evaluations
CE Marking does not cover:

- Comparative data
- Evaluation and validation
- Ease of Use
- Monitoring of internal QCs
- External QA
- Use of combinations
  *ie kits/automated platforms
This figure is based on data generated by testing 21 seroconversion panels in each of the HIV screening tests shown.

= Combined Ag/Ab assay  = immunometric assay  = Class specific antibody capture assay  = antiglobulin assay.
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  - Dr Satnam Sagoo
# Quality Control Reagents we provide

## Current list of reagents for serology

<table>
<thead>
<tr>
<th>Annex II List A</th>
<th>Annex II List B</th>
<th>Non-Annex II</th>
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<tbody>
<tr>
<td>HIV</td>
<td>Toxoplasma</td>
<td>Hepatitis A</td>
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<tr>
<td>HTLV</td>
<td>CMV</td>
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<td>Hepatitis B</td>
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<td>Hepatitis C</td>
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<td>Malaria</td>
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<tr>
<td>HIV p24 antigen</td>
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<td>Influenza H5</td>
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<td>Country</td>
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<tr>
<td>Italy</td>
<td>Ukraine</td>
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</tbody>
</table>
Summary

- NSMs are based on a consensus supported by literature
- Wide consultation
- Led by professionals and consult with recognised experts
- Hosted by HPA
- Supported by NHS and other stakeholders
- Progressive and responsive
- Current and regularly reviewed
- Free!
- Quality if multifactorial!
Evaluations and Standards Laboratory
Acknowledgements

Virology working group
Ken Mutton (chair and editor for Clinical Virology Network)

Medical Editor
Dr Mark Farrington

Members of Working Groups

Department for Evaluations, Standards and Training
All in DEST, especially:
Keith Perry
Ruhi Siddiqui, Jyotsna Vohra, Clare Harris, Russell Croker, Janet Norcup
Ian Sharp
Joe Vincini

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Department for Evaluations, Standards & Training
2009 Joint Symposium
12th—13th March

Respiratory Viruses
‘The Old and the New’

QCRU Users day
‘Quality Control in the Small Laboratory’

Thursday 12th March 2009

Friday 13th March 2009

Contact: Janet Norcup  ☎ +44(0)20 8327 7920  janet.norcup@hpa.org.uk