MICROBIOLOGICAL SCREENING IN ORGAN DONATION – AN OVERVIEW

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TOPICS FOR DISCUSSION

- General concepts
  - Screening
  - Assays
  - Need for confirmation and further testing
  - Results

- Pathogens
  - Viruses
  - Bacteria
  - Parasites

- Terminology and lay out of laboratory reports

- Transcription onto EOS

- Liaising with Laboratories

- Liaising with Transplant Centres
PRE-TRANSPLANT INFECTIOUS DISEASE SCREENING
GENERAL CONCEPTS
Pre Transplant Infectious Disease Screening (1)

Rationale

- Essential for the success of solid organ transplantation
- Identification of conditions which may disqualify donor or recipient
- Identification of conditions that can/need to be treated before transplant
- Defines risk of donor-derived infection
- Determines strategies to mitigate risks to the recipient
- The acceptability of risks for infectious complications after transplantation depends also on the urgency of transplantation of a vital organ as well as the availability of organs
Pre Transplant Infectious Disease Screening (2)

- Screening for an infectious pathogen
  - Look for antigens
  - Look for genomic material (Nucleic Acid Test – NAT)
  - Look for evidence of host immune response against the pathogen
    - IgM
    - IgG
    - Total antibodies (IgG and IgM)

- Laboratories’ approach to screening
  - Different platforms and assays
  - Different protocols and strategies
  - Different reporting format
  - Different reporting mechanisms
SEROLOGICAL ASSAYS (1) - EXAMPLES

Antibody detection

Antigen detection

Signal strength is directly proportional to the concentration of the Analyte
SEROLOGICAL ASSAYS (2)

- Sensitivity
- Specificity

- Confirmatory assays
- Supplementary assays

- Reference tests
- Final report
POSSIBLE CAUSES OF NON-SPECIFIC OR WEAKLY REACTIVE SEROLOGY RESULTS

- Presence of antibodies to cellular components in which the viral antigen was grown
- This is common in multiparous women and in individuals who have had blood transfusions
- The presence of autoantibodies in individuals with autoimmune disease
- The presence of antibodies to some parasitic agents such as malaria
- Infection with another closely-related pathogen
- An individual who is infected with the pathogen and is in the process of seroconversion
ASSAY SENSITIVITY VS ASSAY SPECIFICITY
HOW TO STRIKE THE RIGHT BALANCE?

More false positive results → More unnecessary discard of organs

More false negative results → Increased risk of infection
Best possible informed risk assessment – discuss!
Detectability of Infectivity - Eclipse and Window Periods -

Time Course of Infection

- Infectivity
- Antigen/Genome detection
- Immune response

- Eclipse period
- Antigen/NAT window
- Antibody window
RESIDUAL RISKS

- A donation given in the window period has the potential to cause an infection in the recipient.

- There is a residual risk of transmission despite negative screening results; this depends on different factors.

- Hence the importance of:
  - good history taking
  - general awareness of what constitutes risk
  - interpretation of results in context, never in isolation
  - sharing information with appropriate parties, enabling informed risk assessment

- Unlike in blood donors, calculation of residual risks of transmission is difficult in organ donation.
<table>
<thead>
<tr>
<th>PATHOGEN</th>
<th>ACRONYM/TEST</th>
<th>SEROLOGY TESTS FOR:</th>
<th>IF CONFIRMED POSITIVE, USUALLY INDICATES:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human Immunodeficiency Virus type 1 and 2</td>
<td>HIV 1/2</td>
<td>Anti-HIV-1 and 2 antibodies and HIV p24 antigen</td>
<td>Infected; further tests required</td>
</tr>
<tr>
<td>Hepatitis B Virus</td>
<td>HBV</td>
<td>minimum of two markers are tested for</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HBsAg</td>
<td>Hepatitis B Surface antigen</td>
<td>Infected; further tests required</td>
</tr>
<tr>
<td></td>
<td>anti-HBcore</td>
<td>Anti-HBV core (total antibodies)</td>
<td>current or past infection, further tests may be required</td>
</tr>
<tr>
<td></td>
<td>anti-HBsAg</td>
<td>Anti-HBsAg antibodies</td>
<td>past infection or vaccination, further tests may be required</td>
</tr>
<tr>
<td>Hepatitis C Virus</td>
<td>HCV</td>
<td>Anti-HCV antibodies (and sometimes also core antigen)</td>
<td>current or past infection, further tests required</td>
</tr>
<tr>
<td>Human T cell Lymphotropic Virus type 1 and 2</td>
<td>HTLV 1/2</td>
<td>Anti-HTLV antibodies</td>
<td>Infected; further tests required</td>
</tr>
<tr>
<td>Cytomegalovirus</td>
<td>CMV</td>
<td>Anti-CMV antibodies</td>
<td>Infected</td>
</tr>
<tr>
<td>Epstein Barr Virus</td>
<td>EBV</td>
<td>Anti-EBV antibodies</td>
<td>infected</td>
</tr>
<tr>
<td>Treponema pallidum</td>
<td>Syphilis</td>
<td>Anti-treponemal antibodies</td>
<td>current or past infection, further tests required</td>
</tr>
<tr>
<td>Toxoplasma gondii</td>
<td>Toxoplasma</td>
<td>Anti-toxoplasma antibodies</td>
<td>current or past infection, further tests required</td>
</tr>
</tbody>
</table>
# Factors Governing Screening of Organ Donors

<table>
<thead>
<tr>
<th>Agent</th>
<th>Prevalence in donors</th>
<th>Screening efficient</th>
<th>Transmission rate</th>
<th>Potential damage</th>
<th>Preventative/therapeutic options</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV 1/2</td>
<td>Low</td>
<td>Yes</td>
<td>&gt;90%</td>
<td>High</td>
<td>Limited</td>
</tr>
<tr>
<td>HTLV 1/2</td>
<td>Low</td>
<td>Yes (specificity an issue)</td>
<td>Rate not known transmission very likely</td>
<td>High</td>
<td>Not well established</td>
</tr>
<tr>
<td>HBV</td>
<td>Low</td>
<td>Yes</td>
<td>High</td>
<td>Low-high</td>
<td>Both, limited</td>
</tr>
<tr>
<td>HCV</td>
<td>low</td>
<td>Yes</td>
<td>50-100%</td>
<td>Low-high</td>
<td>Limited</td>
</tr>
<tr>
<td>CMV</td>
<td>Moderate-high to high</td>
<td>Yes</td>
<td>&gt;80% in R-</td>
<td>Low-high</td>
<td>Both</td>
</tr>
<tr>
<td>EBV</td>
<td>High &gt;80% (adults)</td>
<td>Yes</td>
<td>&gt;80% in R-</td>
<td>None-high</td>
<td>Not well established</td>
</tr>
<tr>
<td>Syphilis</td>
<td>Low</td>
<td>Yes</td>
<td>Rate not known transmission very likely</td>
<td>Low</td>
<td>Both</td>
</tr>
<tr>
<td>Toxoplasma</td>
<td>Low-moderate</td>
<td>Yes</td>
<td>Heart ~50% in R-</td>
<td>Low-high</td>
<td>Both</td>
</tr>
</tbody>
</table>

Schaffner A Clin Infect Dis. 2001
Viral infections
CYTOMEGALOVIRUS (CMV)

- Herpes virus family
- Common virus that can infect people of all ages
- After primary infection, CMV establishes life-long latency and can reactivate
- Widely distributed in the general population with seroprevalence ranging from 30 to 97%
- Most healthy adults and children will have no signs or symptoms, and no long-term effects from CMV
- CMV can cause severe disease in certain vulnerable groups
  - Congenital CMV
  - Specific end organ or disseminated disease in the immunocompromised
    - Pneumonitis, hepatitis, colitis, retinitis
CMV IN SOLID ORGAN TRANSPLANT RECIPIENTS

- CMV is by far the most common infection in solid organ transplant recipients
- Major cause of morbidity
- Donor-derived virus can result in
  - primary infection in the immunologically naive recipient
  - superinfection in the previously seropositive recipient
- Reactivation of recipient’s own infecting strain can also occur
- Acquisition of CMV from blood transfusion is no longer a frequent event, particularly with leucodepletion of blood products
D and R CMV serostatus are important predictors of post transplant disease

D+/R- highest risk of tissue-invasive disease and recurrent CMV infection

D+/R- requires strategies to prevent symptomatic infection
- Monitoring with pre-emptive treatment
- Prophylaxis

Seropositive recipient is at risk of CMV reactivation
CMV IN SOLID ORGAN TRANSPLANT RECIPIENTS

- Transmission rate when D+/R- is >80%, with risk being higher amongst recipients of lung, bowel and pancreas compared to liver and kidney recipients.

- All donors and recipients should be tested for CMV infection using commonly available serologic techniques.

- Without some form of prevention, active CMV infection characteristically occurs in the first 3 months following transplant.

- Onset may be delayed in patients receiving CMV prophylaxis.

- CMV surveillance (molecular monitoring for CMV viraemia) can detect virus replication and allow pre-emptive treatment, before occurrence of symptoms and end-organ disease.
CMV SEROLOGY

**CMV IgG**
- **Negative:** no CMV infection of > 4 weeks duration (in immunocompetent subjects)
- **Positive:** infected

**CMV IgM**
- **Positive:** possibly recently acquired or reactivated CMV infection
- CMV IgM persists for 2 – 3 m after a primary infection and can be detected again in the case of a reactivated infection.
- Not always positive in recently acquired or reactivated infection

* The increase in IgM during reactivation depends substantially on the test used
CMV SEROLOGY SCREENING

- The serologic tests most frequently used for donor screening is CMV IgG

- Solid organ donor testing for CMV IgG is regarded as an efficient screening method

- Tests for CMV IgG have better specificity than IgM tests, or tests combining IgG and IgM (i.e. total antibodies)

- The use of IgM for CMV donor screening has been considered but is not commonly used on the basis of the little information gained and the high rate of false positive results
CMV SEROLOGY SCREENING

- The objective of screening is to establish the serostatus of the donor, i.e. whether or not there is serological evidence of previous exposure to CMV.

- Identifying IgG antibodies in the donor gives a 70% – 90% risk that CMV will be transmitted to a seronegative recipient.

- Transplant centres should use either pre-emptive therapy or prophylaxis to reduce the risk of CMV disease.
EPSTEIN BARR VIRUS (EBV)
EBV belongs to the Herpesviridae family and causes a life-long, latent infection of B lymphocytes. EBV establishes a harmless life-long infection in almost everyone it infects and rarely causes disease unless the host–virus balance is upset. More than 90% of the world's population is EBV infected. EBV seroprevalence is very high in the UK adult population, at levels >80%; from a prevalence around 30% in infants aged 1-4 years it increases largely to 72% in 15-19 year olds. Hence recipient and donor serology is more informative in the younger age groups.
EBV

- Generally spread to and amongst young children through salivary contact, mostly asymptomatic.
- If infection is delayed into teens/adulthood, 50% symptomatic.
- Causative agent of Infectious Mononucleosis (glandular fever).
- By influencing B-cell survival mechanisms EBV may induce tumours such as B lymphoproliferative disease and Hodgkin's disease.
- Post-transplant lymphoproliferative disease (PTLD).
EBV SEROLOGY- CURRENT PRACTICE

- There is no requirement for EBV serology to be part of the pre-transplant screening and it is therefore largely available post-transplant.

- EBV serology should be performed on donors and recipients; this is particularly important in paediatric transplantation.

- The highest risk of PTLD is in the D+/R-, but as matching of D-/R- poses its own challenges and problems, this is not routinely applied in practice.

- Knowledge and documentation of EBV serostatus of D/R is good clinical practice, as it informs the overall risk of EBV-related complications and allows modulation of immunosuppression, virological monitoring or other appropriate interventions, according to local protocols.
EBV SEROLOGY

Viral Capsid Antigen = VCA
EBV Nuclear Antigen = EBNA
HUMAN IMMUNODEFICIENCY VIRUS TYPE 1 AND 2 (HIV 1 AND 2)
UK EPIDEMIOLOGY

- An estimated 96,000 (90,800-102,500) people were living with HIV in the UK by the end of 2011.
- The overall prevalence in 2011 was 1.5 per 1,000 population.
- The highest rates reported among men who have sex with men (MSM) (47 per 1,000) and the black African community (37 per 1,000).
- Rates of new HIV diagnoses and HIV prevalence continue to be significantly higher in London than elsewhere in the UK. The city contains 18 of the 20 local authorities with the highest prevalence of HIV infection.
IMPROVEMENT OF LIFE EXPECTANCY WITH ANTI-RETROVIRAL TREATMENT

HIV patients aged 20, who started antiretroviral treatments 16 years ago, could expect to live to an average age of 50

Life expectancy jumps to 66 for subjects in the same group who began their treatment just 4 years ago

The sample was made up of 17661 adults aged 20 and over who received cART in between 1996 and 2008 in the UK

Natural course of untreated HIV infection

Hence it is important to make a diagnosis to allow appropriate management!
SEROLOGICAL DIAGNOSIS/SCREENING

- HIV reactive result in screening assay needs to be tested by alternative tests for confirmation

- Molecular tests can be used to detect
  - HIV RNA in plasma (viral load)
  - HIV Proviral DNA in peripheral blood mononuclear cells (PBMCs)

- According to a pre-determined criteria, the final result may be
  - Indeterminate
  - Positive
  - Negative
HEPATITIS B VIRUS – HBV
Global Patterns of Chronic HBV Infection

- >1/3 world population has been infected at some point
- Approximately 350 million people are currently infected
- High Prevalence (>8%): 45% of global population
  - lifetime risk of infection >60%
  - early childhood infections common
- Intermediate Prevalence (2%-7%): 43% of global population
  - lifetime risk of infection 20%-60%
  - infections occur in all age groups
- Low Prevalence (<2%): 12% of global population
  - lifetime risk of infection <20%
  - most infections occur in adult risk groups
PREVALENCE OF HBV AND INCIDENCE OF HEPATOCELLULAR CARCINOMA (HCC)

**World prevalence of HBV carriers**
- HBsAg carriers – prevalence
  - <2%
  - 2–7%
  - >8%
  - Poorly documented

**Annual incidence of primary HCC**
- Cases/100,000 population
  - 1–3
  - 3–10
  - 10–150
  - Poorly documented

WHO 1999
Hepatitis B Transmission – BLOOD BORNE VIRUS

- Heterosexual Sex
- Homosexual Sex
- Mother to Child
- Sharing Needles (and equipment)
- Receipt of Blood Products
- Needlestick Injury
Patients with Acute Hepatitis B who Report Selected Epidemiologic Characteristics, by age group, U.S. 2007

- Injection Drug Use: 15%
- Sexual Contact with Hepatitis B Patient: 6.2%
- Household Contact of Hepatitis B Patient: 2.3%
- Homosexual Activity (male): 10.5%
- Medical Employee with Blood Contact: 0.6%
- Hemodialysis: 0.2%
- Had > 1 Sex Partner: 38.3%
- Blood Transfusion: 0.5%
- Surgery: 11.7%
- Percutaneous Injury (e.g. needlestick): 4.3%
- Unknown: 58.0%

*The percentage of cases for which a specific risk factor was reported was calculated on the basis of the total number of cases for which any information for that exposure was reported.

Natural History of HBV Infection

Early Childhood HBV Infection:
- 90% Chronic Infection
  - Inactive Chronic Infection
  - Chronic Active Hepatitis
    - Up to 25% Develop Cirrhosis
    - Up to 5% Develop Liver Failure or Liver Cancer
- 10% Infection Resolved

Adult HBV Infection:
- 5% Chronic Infection
  - Inactive Carrier
  - Chronic Active Hepatitis
    - Up to 15% Develop Cirrhosis
    - Up to 5% Develop Liver Failure or Liver Cancer
- 95% Infection Resolved
**Hepatitis B – Prevention**

- **Vaccination (pre and post exposure)**
  - highly effective recombinant vaccines
  - given to those at increased risk of HBV infection (e.g. healthcare workers, haemodialysis patients)
  - also given routinely to neonates as universal vaccination in many countries

- **Hepatitis B Immunoglobulin - HBIG**
  - post-exposure prophylaxis

- **Other measures**
  - screening of blood donors, blood and body fluid precautions.
HEPATITIS B VIRUS
DANE PARTICLE AND GENOME

- HBsAg
- HBcAg
- Partially double-stranded DNA
- DNA Polymerase
- HBeAg (circulating form)

- Partially double-stranded circular DNA virus
- A member of the Hepadnaviridae
HBV SEROLOGY — ACUTE INFECTION, WITH RECOVERY

<table>
<thead>
<tr>
<th>Incubation</th>
<th>Acute Infection</th>
<th>Early Recovery</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>(4 – 12 weeks)</td>
<td>(2 weeks – 3 months)</td>
<td>(3 – 6 months)</td>
<td>(6 – 12 months) (Years)</td>
</tr>
</tbody>
</table>

**Symptoms**

- *HBsAg*
- HBeAg
- Anti-HBc IgM

**Relative Concentration**

- **Anti-HBc Total**
- **Anti-HBs**
- **Anti-HBe**

**HBsAg** = infection

**Anti-HBcore antibodies** = exposure to HBV at some point

**Anti-HBsAg** = immunity
HBV SEROLOGY PROFILE — CHRONIC CARRIER

**HBsAg** = infection
Anti-HBcore total antibodies = exposure to HBV at some point
EPIDEMIOLOGY - WORLDWIDE

- **HCV Pandemic**
  - Estimated 3% of global population infected
  - 5 times as widespread as HIV-1
  - 4 million new infections per year

- Most frequent cause of chronic hepatitis, cirrhosis and hepatocellular carcinoma in the USA and most Western nations
Global burden of HCV infection

**Western World**
0.5-1.8% prevalence
  - USA 1.8%
  - UK 0.5%
  - 80% IVDU

**Egypt**
Highest prevalence in the world
  - 20% prevalence
  - 90% health-care associated
  - (vaccination <1986)

**Eastern Europe**
5-7% prevalence
  - 60% health-care associated
  - 40% IVDU

MODES OF HCV TRANSMISSION IN AREAS OF LOW ENDEMICITY

- Injecting drug use 60%
- Sexual 15%
- Transfusion 10% (before screening)
- Occupational 4%
- Other 1%*
- Unknown 10%

Risk of heterosexual transmission between monogamous partners is low

* Nosocomial; iatrogenic; perinatal

Source: Centers for Disease Control and Prevention
HEPATITIS C VIRUS

- Flavivirus, RNA
- 9.5 kb genome
- Identified in 1989
- 6 major genotypes
- > 20 subtypes
- Multiple quasispecies
NATURAL HISTORY OF HCV INFECTION

Infection by Hepatitis C Virus

Acute Hepatitis
(>90% Asymptomatic)

Spontaneous recovery
(15-25%)

Chronic hepatitis
(75-85%)

Asymptomatic
(80%)

Chronic active
(20%)

Treatment

Cirrhosis
25%

Transplantation

HCC

6 Months

10-30 Years
Detection of HCV IgG in serum

Detection of HCV core Antigen in serum (presence of virus)

Detection of HCV RNA in serum or plasma (presence of virus)

Confirmation of viraemia following positive antibody test

Assessment of treatment response

Suspicion of recent infection with negative serological results

In patients with known reasons for false negative results on antibody testing
Serologic Pattern of Acute HCV Infection with Recovery

Symptoms +/-

Time after Exposure

Titer

anti-HCV

Symptoms +/-

HCV RNA

ALT

Normal

0 1 2 3 4 5 6 1 2 3 4

Years Months

Time after Exposure
Serologic Pattern of Acute HCV Infection with Progression to Chronic Infection

Symptoms +/-

Time after Exposure

Titer

anti-HCV

HCV RNA

ALT

Normal

0 1 2 3 4 5 6 1 2 3 4

Years Months
There are assays that detect both HCV antigen and antibody.

They are called “combo” (=combination) assays and are more sensitive than the antibody only tests.

There also assays that test for HCV antigen separately.

Any lab performing these will report antibody and antigen separately.
HUMAN T CELL LYMPHOTROPIC VIRUS TYPES I AND II (HTLV I AND II)
HTLV

- HTLV types I and II are transmissible through breast feeding, sexual contact, exposure to blood, and injecting drug use.
- HTLV-I is endemic in the Caribbean, Japan, South America and parts of Africa.
- HTLV-II is found among some native American groups and injecting drug users.
- An infected individual's lifetime risk of developing symptomatic disease is low (less than 5%).
- HTLV-I infection may cause adult T cell lymphoma (ATLL), HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) and other inflammatory conditions.
- There is some evidence that HTLV-II infection is also associated with neurological and lymphoproliferative disorders.
• HTLV is endemic in certain areas (especially SW Japan, the Caribbean and parts of Africa and South America) where up to 10% or more of the population may be infected
• In the UK, HTLV-1 was estimated to cause around 25 cases of acute T-cell leukaemia/lymphoma in 2010
BACTERIA
Treponema pallidum (Syphilis)
TREPONEMA PALLIDUM

Gram-negative spirochaete bacterium with subspecies that cause treponemal diseases such as syphilis, bejel, pinta and yaws.

<table>
<thead>
<tr>
<th>T. pallidum subspecies</th>
<th>Disease</th>
<th>Transmission</th>
<th>Effective treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>pallidum</td>
<td>syphilis</td>
<td>Sexual; vertical; blood/tissues/organs</td>
<td>yes</td>
</tr>
<tr>
<td>pertenue</td>
<td>yaws</td>
<td>Non-venereal</td>
<td>yes</td>
</tr>
<tr>
<td>carateum</td>
<td>pinta</td>
<td>Non-venereal</td>
<td>yes</td>
</tr>
<tr>
<td>endemicum</td>
<td>bejel</td>
<td>Non-venereal</td>
<td>yes</td>
</tr>
</tbody>
</table>

Yaws: transmission by skin-to-skin contact, seen in tropical, hot and humid geographical regions.
Syphilis

- Syphilis is transmitted from person to person by direct contact with syphilis sores
- Syphilis is also transmitted vertically (maternal-foetal infection)
- Transmission via blood products and organ transplantation
- Disease in adults
  - Primary, secondary, late and latent stages
  - About 15% of people who have not been treated for syphilis develop late stage syphilis, which can appear 10–30 years after infection began
- Penicillin is the treatment of choice
**Syphilis Serology**

- Reactive screening test must be followed by further testing to allow interpretation of serostatus.
Toxoplasma

- *Toxoplasma gondii* is an ubiquitous parasite which can infect all mammal and bird species throughout the world.

- Up to one billion people have been exposed to the parasite worldwide, but seroprevalence varies widely between countries.

- Infection can occur via:
  - Ingestion of oocysts or tissue cysts
  - Vertical transmission (congenital)
  - Blood/tissues/organs

- UK seroprevalence is estimated at 7-34%.

- Following the acute active stage of the infection the parasite persists in the body for many years or decades in the form of cysts, particularly in heart and skeletal muscle and nervous system tissues.

- In immunocompetent persons these cysts are considered not to pose a health risk.
TOXOPLASMA IN THE IMMUNOCOMPROMISED

- Can occur as a result of reactivation of chronic infection, or primary infection and may be very severe
- Donor derived infection in SOT
  - risk is high in cases of organs that are recognized sites of encystation of the parasite, e.g. the heart
  - markedly lower in other SOT recipients
  - Clinical symptoms usually occur within the first 3 months after transplantation, sometimes as early as 2 weeks post transplant
    - febrile myocarditis, encephalitis or pneumonitis
- The nature and severity of symptoms varies according to the cause and degree of the immunosuppression
- The CNS is usually affected, but reactivation can also cause a range of systemic symptoms, including pneumonitis
TOXOPLASMA IN THE IMMUNOCOMPROMISED

- Donor seropositivity is not a contra-indication for donation of solid organs
  - Recipient prophylaxis is efficient
  - Routine prophylaxis with trimethoprim-sulfamethoxazole for Pneumocystis infection is routinely used in SOT recipients and is effective against toxoplasma
TOXOPLASMA - DIAGNOSIS

- Diagnosis of toxoplasmosis is complicated by the fact that the parasite can be present in an acute, chronic, latent or reactivated form

- A combination of serological tests is frequently required to establish whether an individual has been more likely infected in the distant past or has been recently infected

- In patients who are immunocompetent, initial tests are carried out to detect IgG and IgM antibodies

- In those who are immunocompromised diagnosis usually involves direct detection of the parasite either by microscopy, parasite culture, and/or nucleic acid detection