Etiological agents and Laboratory diagnosis of viral infections of skin

Skin & Locomotor Module- - *21.09.2018*

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Laboratory diagnosis of virus infections – General guidlines

Principle for optimal selection of specimens for virus diagnosis - four basic questions to answer

(1).Where is the virus at this stage of illness?

(2). Can virions, Ags, NA be detected from the lesion site?

(3). Can the virus be grown readily in cells? *i.e. HSV, enteroviruses (yes)*

VZV, HBV (no)

(4). what are kinetics of Ab response? *i.e. is it necessary paired sera or single sera for IgM*

Specimen collection for virology:

Timing of collection is critical in acute infections

Choice of specimens is critical in acute, chronic and sub acute infections

if disease express on surface get from surface i.e. respiratory, diarrhoea, skin vesicles

If deep or generalized disease get from multiple sites i.e. non-vesicular rash, meningitis, encephalitis, PUO

Type of specimens for virus diagnosis

Swabs from lesions: should not be dried, head broken into VTM. *i.e.skin, throat*

Scrapings of lesions to get infected cells, *i.e. base of vesicles, corneal ulcers*

Aspirates of secretions or exudates, i.e. *post nasopharyinx, conjunctivial, cervical*

Excreta: i.e, *urine, faeces*

Biopsies: *i.e. needle aspirates, open exploration, endoscopy*

Peripheral blood*: i.e., preservative free heparin bottles, buffy coat taken by ficoll gradient*

Serology: *i.e. 5ml blood into sterile bottle*

NPA (nasopharyngeal aspirate):

- Aspirate secretions from nasopharyinx with a feeding catheter size 8 attached to a mucous extractor (suction machine or ward suction line or 20ml syringe)
- Tip of catheter pass through nose for 7cm & suction applied. Aspirate is washed into the mucous trap with VTM.

Biopsy (lung, liver, kidney, LN):

- Tissue into dry sterile container
- Bring to lab at once; part of the specimen fix for histology, part for frozen sections, part emulsify in a tissue grinder with VTM for virology
- Liver: for HBV snap frozen.

<u>Transport of specimens</u>

- VTM (viral transport medium): 3 main components
  - 1. Buffering system to maintain physiological pH.
  - 2. Low concentration of protective protein *(albumin, gelatin or Foetal calf serum/FCS)*
  - 3. Antibiotics & antifungals

i.e., Cell culture maintenance medium (DMEM with 1% FCS + antibiotic & antifungals . 2-4ml in one bottle is sufficient

Skin manifestations due to virus infections

- **Maculopapular rashes**
- **Blisters /vesicular rashes**
- **Ulcers**
- **Warts**
- **Cancers**

Maculopapular rash: differential diagnosis

Allergy to food or drugs

Insect bites or stings

drug adverse reactions i.e. antibiotics

Chemotherapy

Scarlet fever

Viral exanthemas/ Maculopapular rashes

Enterovirus and echovirus infections

Fifth disease (erythema infectiosum) - parvovirus infection

Roseola infantum (sixth disease) – human herpes virus 6 or 7 infection

EBV infection

CMV infection

Rubella (German measles)

Rubeola (Measles)

Acute HBV infection

Acute HCV infection

HIV - Seroconversion exanthema

Dengue viruses type 1-4

Virus infections of skin causing Blisters/ Vesicular lesions

(1). Herpetic whitlow: Infection fingers with HSV, i.e. healthcare workers

(2). Orf: Infection with Orf virus i.e. sheep or goat farmers

(3). Hand foot and mouth disease: Enteroviruses - Coxsackie A virus infection usually in a child

Viral etiology of skin diseases

1. Measles virus
2. Rubella virus
3. Parvovirus
4. Herpes viruses:
5. Pox viruses:
6. Papilloma viruses
7. Enteroviruses

Measles:

- Morbiliform **rash**

- Fever,

- Runny nose,

- Unproductive cough,

- No lympadenopathy .

Complications of measles include;

- giant cell pneumonia,
- encephalitis
- SSPE

Etiology: Family of Paramyxoviridae. Genus: Morbillivirus,

Human pathogens: measles virus (rubeola)

Transmission: droplet spread of respiratory secretions.

Virus entry: Through upper respiratory tract or conjunctiva

Incubation period: 10-14 days, leucopenia develop as virus replicates in lymphoid tissue.

Prodromal illness: 2-4 days

Morbiliform rash: Maculopapular rash develop over 2-3 days then fades.

Infectious period: from prodromal stage and for 4 days after onset of illness.

Treatment: No specific antiviral treatment

Prevention: vaccination. Refer to EPI of Sri Lanka

Differential diagnosis: Measles, rubella, roseola infantum

Complications of measles:

(i). Most complications due to **secondary bacterial infection** of necrotic epithelial surfaces of URT, and include

- Bronchitis
- Bronchiectasis
- Otitis media
- Purulent conjunctivitis
- Laryngotracheitis

(ii). CNS complications include

- Acute measles post infectious encephalitis
- Subacute sclerosing panencephalitis (SSPE)

(iii). Giant cell pneumonia – often fatal due to direct virus spread to LRT. Occurs usually in patients with underlying illness e.g. leukaemia

Diagnosis of measles

Clinical

By noting **Koplik's spots** on buccal mucosa.

Small grayish white lesions are present during prodromal stage but fade once the rash appear.

Accuracy improves

if rash for at least 3 days

if fever at least for 1 day

if at least one of the symptoms out of cough, coryza or conjunctivitis

Laboratory confirmation of measles

(1). Direct antigen detection by IF test in Nasopharyngeal aspirates (NPA)

(2). Serology:

- Single or paired sera
- Detection of measles IgM in SSPE & confirm by detecting Ab in CSF

(3). Virus isolation from the throat (done rarely)

Measles virus CPE; Very large syncytia /giant cells can be formed during replication of measles virus in cell culture. Presence of Intracytoplasmic eosinophilic inclusions

Rubella virus & Parvovirus B19

Both viruses cause really trivial childhood infections; similar, **mild rash diseases .** But they all have interesting complications.

- **arthriti**s is a common complication of both.
- Both may endanger the **foetus** if the mother is infected in pregnancy

Rubella

Most common in children of 4-9 yrs

Fever, malaise,

Irregular **macularpapular rash** lasting for 3 days. (Immunopathology involved (Ag - Ab complexes)

Enlarged LNs seen behind the ear (post auricular & sub-occipital).

Arthritis,

Intrautrine infection →congenital malformation, progressive panencephalitis)

Diagnosis of rubella

Clinical diagnosis: some times possible. But unreliable, because symptoms are fleeting, can be caused by other viruses, rash is not diagnostic.

Lab diagnosis; 1. Isolation of virus.

2. Sero-conversion.

Rubella virus isolation : rarely indicated

From throat

No CPE. Therefore, indirect methods (i.e. IF test) needed to demonstrate virus

Serology of rubella:

Rubella specific IgM Abs by ELISA OR,

Rising titre of Abs (IgG)/whole Ab equal to or more than 4 fold rise by HI, ELISA, CF, RIA (over 10 days). Serology is important as sub clinical infection is common and rash mimics other virus infections.

Erythrovirus B19/Parvovirus B19

Spread by the respiratory route (droplet infection), and the rash appears after about 17 days.

The disease it causes, **erythema infectiosum**

It is also **"fifth disease"** or **"slapped cheek disease/rash/syndrome**" because of the facial rash.

Parvo B19's site of replication **is red cell precursors** in the bone marrow. No specific treatment or vaccine

Parvovirus B19 infections

1. Aplastic crisis in anaemic individuals

2. Arthropathy in adults

3. Intrauterine infection : foetal death, hydrop foetalis

4. Persistent infection in immunodeficient individuals

5. Symptom-less infection is common.

Laboratory diagnosis of Parvovirus B19: Laboratory diagnosis is essential to exclude rubella.

Based on the presence of IgM antibodies. Detectable at the time of rash & for 2-3 months

The virus cannot be cultivated in routine cell culture lines, but direct detection of the viral DNA may be achieved by PCR & DNA hybridisation (dot blot in serum).

Herpes virus infections of the skin :

(a**). HSV** (herpes labialis, herpes genitalis, herpetic whitlow)

(b). **VZV** (varicella, shingles)

(c). **HHV6** & 7 (Rash = Roseola infantum /Exanthem subitum/6th disease)

(d). **HHV 8** (Kaposi's sarcoma)

Orolabial herpes - HSV 1 & HSV 2

Painful mouth and sore throat, unable to eat properly. Number of **vesicles** seen on plate, tonsils, tongue.

Causative agent: HSV type 1 or 2. Herpesvirus particles : EM appearence

Age: primary infection usually in children, but can be seen in older individuals.

HSV -1 Primary disease:

Vesicles become ulcerated leading to extensive acute ulcerative gingivostomatitis.

Spread to adjacent skin

Systemic upset, with fever and cervical lymphadenopathy.

2-3 weeks before cessation of new lesions.

Infection by HSV – 1 (Herpes simplex virus type 1) type is common. Most common in upper parts of body. Some times in children Infection may passed unnoticed

HSV -1 Secondary/recurrent /cold sores:

Due to reactivation of latent virus in trigeminal ganglion

Much milder disease. i.e. Painful, burning and itching lesions -few lesions, unilateral. Crops of vesicles appear at mucocutaneous junctions of mouth, nostrils. Dangerous form is reactivation from ophthalmic branch of 5th Nerve can lead to Severe kerato-conjunctivitis Visual impairment

Herpetic whitlow: Infection fingers with HSV

- **Painful,** Recurrences may occur
- Occupationally associated (many healthcare workers i.e. dental workers, needle stick injuries)
- Auto-inoculation of nail folds in children who bites their nails

Varicella (chicken pox)

Clinical course of chicken pox

Incubation period: 13-17 days

Rash usually appears first on the trunk, then spread to involve face, scalp and limbs. Rash is itchy in some but not all patients

Lesions evolves from **maccule → papule →** to **vesicle in a few hours → to crust in 4 days**.

Usually several lesions in the mouth, pharynx and conjunctiva may be involved

Cropping occurs over several days

Some generalized lymphadenopathy may occur

Diagnosis of varicella:
Clinical:

Essentially a clinical diagnosis

The florid vesicular rash is characteristic of chicken pox

In the early stages or if the rash is less profuse, other possibilities would include HSV infection or drug reaction

Laboratory diagnosis:

Laboratory diagnosis is done if necessary to confirm.

Demonstrate virus in vesicle fluid by EM or antigen detection

Isolation of virus from vesicle fluid or

By demonstrating a **rise in antibody titre** in paired serum samples

Shingles or Herpes Zoster

"Shingles" comes from latin cingulum, which means girdle or belt.

It occurs in an area of the skin that is supplied by the sensory fibers of a single nerve- dermatome. Rash appears as well-defined band on one side of body, or on one side of face, arms or legs.

About 20 % of those people who have had chicken pox will get zoster at some time during their lives. Most people will get zoster only once.

Risk factors for the development of herpes zoster:

(1). Increasing age. Presumably reflecting declining immunity to VZV

(2). Trauma (including surgery)to an area of the body →zoster in that area some days later.

(3). Stress

(4). Immunodeficiency or suppression results in increased frequency of reactivation;

Blood stream →generalized zoster rash & life threatening involvement of internal organs, e.g. lung, liver

Predisposing factors for severe zoster:

1. HIV infection
2. Malignancy, especially of the reticular endothelial sytem, e.g.
3. Chronic lymphocytic leukaemia
4. Multiple myeloma
5. Hodgkin's and non-Hodgkin's lymphomas
6. Immunosuppression for transplant recipients
7. Chemotherapy
8. High dose steroid therapy
9. Radiotherapy

Roseola infantum (Exanthem subitum/6th disease)

Causative agent: primarily by human herpes virus-6 and less commonly by HHV-7**.**

Age: Mostly in 6 months to 2 years old

Clinical features:

A sudden high fever that lasts for 3-5 days is an early feature of roseola.

When the fever disappears, a rash appears. The rash is not contagious.

The incubation period is 9 to 10 days.

Kaposi's sarcoma (KS)

Purplish lesions of a skin cancer

Kaposi,s sarcoma is common among AID patients.

KS is caused by Human herpes virus-8 /HHV-8 /Karposis sarcoma herpes virus (KSHV)

Enterovirus infections of the skin:

(a). vesicular rash

(1). Coxsackie A viruses,

(2). Enterovirus 71

(b). Generalized exanthema : (maculopapular rash)

(1). Coxsackie B viruses,

(2). Ehcoviruses

Hand, Foot, and Mouth Disease

Due to infection with Coxsackie A viruses, & Enterovirus 71

Painful blisters on: Throat, Tongue, Gums, Cheeks, Palms of hands, Soles of Feet

Faeco-oral transmission

Hand hygiene is importanrt

Pox virus infections of the skin

Small pox virus (small pox)- eradicated viral disease

Contagious pustular dermatitis virus /Orf virus (CPD/Orf)

Molluscom contagiosum virus (Molluscom contagiosum)

Parapox viruses (parapox)

Parapox viruses

1. Widespread in sheep, goats and cattle.
2. Lesions in humans are essentially same but go under different names. Naming is based on the identity of the host from which the infection was acquired.

Parapox viruses and diseases	
Mode of spread	***Resulting human disease***
Cattle to humans	Pseudocowpox, paravaccinia or milker's nodes
Sheep or goats to humans	Orf or contagious pustular dermatitis (CPD)

Contagious pustular dermatitis (CPD/Orf)

Zoonotic infection acquired from sheep or goats caused by a pox virus

Etiology: CPD virus (Orf virus)

Painless lesions (diagnostic feature)

Re-infections may occur

Occupationally associated (Occupational hazard of farm and slaughter house workers and veterinarians).

Constitutional upset is slight

Host immune response to infection is poor

Recurrent lesions due to **re- infection** (not reactivation) may occur

Pathogenesis Orf

Erythematous papules → progressed into red centre surrounded by white halo and outer inflamed halo (**target stage**) → proceeds to a **nodular stage (**which may have a weeping surface) - last a week → **heals**

Diagnosis of Orf:

Usually based on the history

Laboratory confirmation by

Demonstration of pox virus particles by electron microscopy

Specimen: fluid exuded from the nodule)

Molluscum Contagiosum

Infect epidermal cells form fresh lesions

Often with umbilicated center (cup shape lesion)

Only infect human

Often with axilla & trunk

Spread by contact. In case of genital lesions – sexually.

Particles can be seen by EM

Smallpox (Variola) - Eradicated disease

Smallpox is an ancient disease, documented as endemic at least 2000 years ago.

Two types

Variola major (severe)

Variola minor (mild)

Clinical Features

Incubation period : 12 days

Started with malaise , vomiting, headache & fever

2-3days - **skin Rash**

2-5 days - single crop of eruptions

Eradication of SmallPox

1970 Incidence of Smallpox less.

1971 Last case in South America.

1975 Last case in Asia. (Bangladesh)

1977 Last case in the world.(Somalia)

Small pox has now been eradicated- the last naturally occurring outbreak of smallpox was in Somalia on 26th October 1977.

First planned extinction of a human virus - *Eradication of smallpox, the terrible human plague in the 20th century*

Lab diagnosis of SmallPox : Eradicated disease. Therefore, important to know inform authorities.

See differentiation with VZV

Collection of specimen from lesions

Demonstration of virus : Light microscope & Electron microscope

Viral antigen by serology : PIG, CF, HA, IF

Isolation of virus from throat washings, skin lesions, blood

Human papilloma virus (HPV) – wart virus

Classification of HPV:

ds DNA genome

Papovavirus family (**pa**pilloma-**po**lyoma-**va**cuolar) contain three distinct genera of viruses.

Papilloma viruses further classified into **types** based on the degree of DNA sequence homology of virus genome – over 60 that infect humans

Anatomical sites of HPV infection:

HPV infect and replicate in squamous epithelium on both keratinsed and mucousal surfaces. Infections are extremely common.

Clinical presentations may be; Cutaneous, Mucosal **or** Anogenital

Cutaneous warts:

Most frequent in childhood and early adolescence due to **HPV type1-4**

Hands (verruca vulgaris)

Feet (verruca plantaris or plantar warts)

May occur elsewhere i.e. face

Mucosal warts:

Single papillomas may occur in mouth, at any age, and rarely recur after surgical excision

Multiple papillomas associated with **type 6 & 11;** commonest benign epithelial tumors of the larynx. More frequent in children than adults and frequently recur after surgical incision

Anogenital warts:

An increasingly common STD

Benign warts (condylomata accuminata); may occur on the penis, vulva, cervix, perianal areas due to **HPV 6 or 11**

Malignant transformation in the cervix associated with **HPV types 16 & 18 (and others)**

Diagnosis HPV lesions

Clinical diagnosis

Laboratory diagnosis:

Biopsy and genome analysis of HPV

Evaluation of women with HPV lesions of the cervix detection of cytologic abnormalities in Cervical smears

Colposcopic examination

Biopsy of suspicious lesions

Biopsy: Screening women for the presence of HPV DNA in premalignant and malignant lesions by PCR is more sensitive than cytology.

Women with genital warts and female partners of males with genital warts should be advised to give regular cervical smears

Flaviviruses: Dengue viruses type 1-4 : **Fever, & Myalgia,**

3-5th day, maculopapular –rash. Rash is immune-complex mediated

+/-haemorrhage