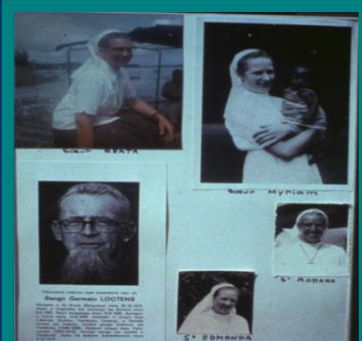


Viral Haemorrhagic Fevers and the Laboratory

Dr Mike Catton

VIDRL

Viral Haemorrhagic Fever (VHF)



- VHF is a clinical syndrome of fever and bleeding diathesis
- Diverse viral causation
- High (albeit variable) mortality rates
- Limited preventative and therapeutic options
- Many have actual or potential human to human transmission risk
- Zoonotic reservoirs, some cryptic
- Exotic to Australia

Haemorrhagic Fever Viruses

Family	Genus	Virus	Distribution	Incubation period (d)	Mortality (%)
Filoviridae	Filovirus	Ebola	Africa	2-21	50-90
		Marburg	Africa	2-14	23-70
Arenaviridae	Arenavirus	Lassa	West Africa	5-16	15-20
		New World Viruses	S. America	7-14	15-30
Bunyaviridae	Nairovirus	CCHF	Africa, Central Asia, E. Europe, Middle East	2-12	10-64
	Phlebovirus	Rift Valley Fever	Africa, Saudi Arabia, Yemen	2-6	<1
	Hantavirus	Hantaan etc	Asia, Balkans, Europe, Eurasia	4-42	1-7
Flaviridae	Flavivirus	Dengue	Asia, Africa, Pacific Americas	2-27	0.5-1
		Yellow Fever	Africa, Tropical Americas	3-6	20
		OMSK HF	Central Asia	2-9	0.5-10
		Kyasanur Forest Disease	India	2-9	3-10

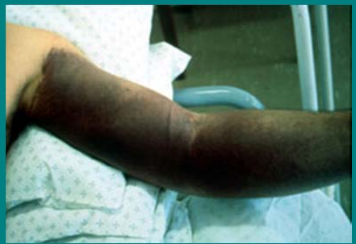
Transmission of VHF Viruses

	Mosquito-borne	Tick-borne	Rodent Borne/ Other	Person-to-person *	Aerosol	BSL①
Arenaviridae						
Lassa fever (LHF)			●	+	+	4
Argentine HF (Junin)			●	+	+	4
Bolivian HF (Machupo)			●	+	+	4
Brazilian HF (Sabia)			●	+	+	4
Venezuelan HF (Guanarito)			●	+	+	4
Bunyaviridae						
Crimean-Congo HF (CCHF)		●	●	+	+	4
Hantaan*			●	+	+	3
Rift Valley Fever (RVF)	●		Livestock	-	+	3
Filoviridae						
Ebola (EHF)			③	+++	+	4
Marburg (MHF)			③	+++	+	4
Flaviviridae						
Dengue, Type 1-4	●			-	-	(3)
Yellow Fever (YF)	●			-	+	3
Kyasanur Forest Fever		●		-	+	3
Omsk Haemorrhagic fever		●		-	+	3
Togaviridae						
Chikungunya (CHF)	●			-	-	3

*Person to person
spread
n= none
o= occasional
f= frequent

① Biosafety level
② Domestic animals
③ Unknown reservoir and source
④ Squirrel, monkey

Clinical Features of viral haemorrhagic Fevers



- Non-specific onset
- Fever, myalgias, arthralgias, headache
- Pharyngitis
- Conjunctival injection
- Gastrointestinal symptoms
- Deterioration or improvement in second week
- Haemorrhagic phenomena (GI, mucosal bleeding, petechia/echymoses)
- Encephalopathy (arenaviruses)
- Hepatitis (arenaviruses, CCHF)
- Multi-organ failure, shock

Clinical Features that differ between viral haemorrhagic fevers

Feature	Lassa Fever	Marburg & Ebola Viruses	CCHF
Usual incubation	7-18 days	4-10 days	2-9 days
Onset	Gradual	Abrupt	Abrupt
Rash	Uncommon	Usual	Absent
Pharyngitis	+++	++	+
Capillary leak	++	+	+
Oedema/effusions	++	+	-
Haemorrhage	+	++	+++
DIC	-	+	+
Pancreatitis	-	+	-
Jaundice	-	±	+
Deafness	+	-	-
Orchitis	-	+	-
Uveitis	-	+	-
CCHF: Crimean-Congo Haemorrhagic Fever DIC: Disseminated intravascular coagulation Key: + = mild; ++ = moderate, +++ = Severe			

History of VHF Diagnostic Capability in Australia



- National High Security Quarantine Unit (NHSQU) commissioned 1982 at Fairfield Hospital
- Centralised model for Quarantineable VHF patient clinical care
- 'Aero-medical evacuation' of patient to NHSQU
- Specific viral diagnostics and clinical pathology in PC4 laboratory (NHSQU)

History of VHF Diagnostic Capability in Australia



- Revision of centralised care model in 1990
- Consistent with revised CDC guidelines 1988
- Designated state isolation units for patient clinical care
- Clinical pathology in designated state hospital pathology departments
- Specific viral diagnostics in NHSQL.

Specific Viral Diagnosis of VHF

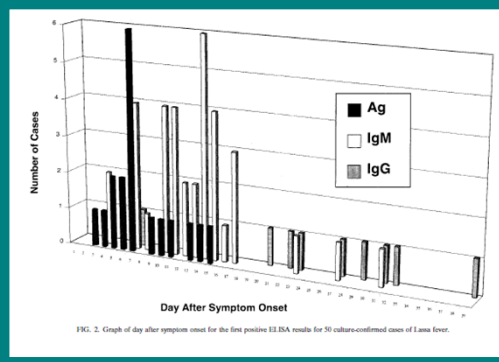
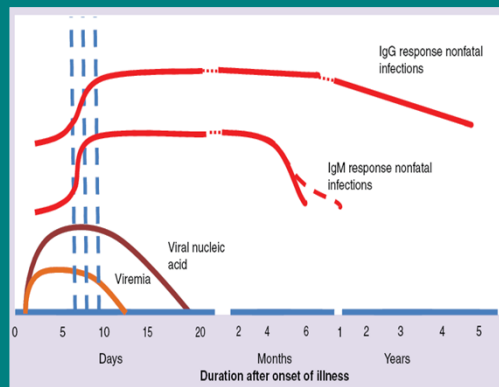
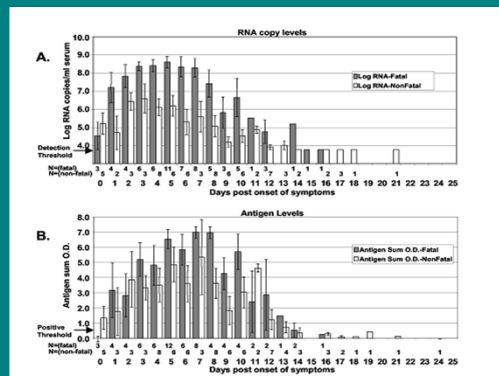
Samples:

- Viruses present in high titre in plasma
- Other body fluids, tissue, swabs may also be tested
- Fatal cases generally die before making a detectable immune response

Diagnostic Assays

- Acute causes
 - Direct virus detection
 - RT-PCR (sensitive and rapid)
 - Antigen detection (less sensitive)
 - Electron microscopy (filoviruses especially)
 - Virus culture (sensitive, slow, BSL-4)
- Convalescent cases
 - serology
 - Classical assays (CFT, NT, AIA limited utility)
 - IFA & EIA (detect IgM & IgG)
 - Native Ag hard to source
 - Recombinant Ag becoming available

Specific Viral Diagnosis of VHF



Towner J et al (2004) J. Virol 78:4330
 Burt F (2011) Future Virol 6:831
 Bausch D (2000) J Clin Microbiol 38:2670

Filoviruses, Marburg + Ebola viruses

- RT-PCR targeting L gene or NP gene
- Ag Capture EIA targeting NP, VP40 or GP
- Native Ag IFA serology
- Recombinant rNP EIA serology
- Cell Culture Vero E6 with IFA or PCR confirmation

Crimean Congo Haemorrhagic Fever Virus

- RT-PCR targeting NP gene
- Ag detection targeting NP gene
- Native Ag IFA serology
- Recombinant NP Ag IFA serology
- Mouse Brain suspension Ag EIA serology
- Recombinant Np Ag EIA serology
- Cell culture Vero E6 with IFA or PCR confirmation

Lassa Virus

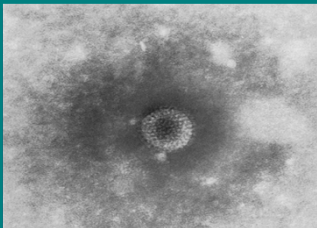
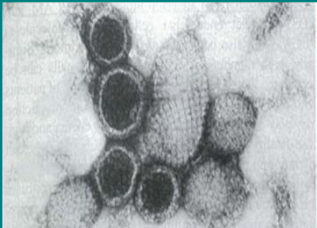
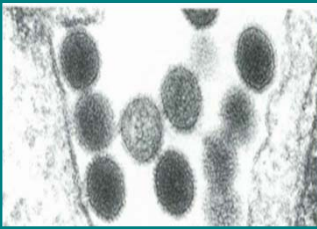
- RT-PCR targeting GP gene
- Ag detection targeting NP gene
- Native Ag IFA serology
- Native Ag EIA serology
- Cell culture Vero E6 with IFA or PCR confirmation

Specific Viral Diagnosis of VHF at the NHSQL



Contents removed at the author's request

VHF Samples in the Laboratory



- VHF viruses predominantly Risk Group 4 agents; some Risk Group 3
- Virulent and therapeutic options are limited
- Viruses can reach high titres in blood/body fluids ($\leq 10^8$ pfu/ml Ebola)
- All viruses except dengue & chikungunya have transmitted by aerosol
- All viruses except Guanarito have caused laboratory infections
- Ebola, Marburg, Lassa & CCHF have greatest secondary transmission potential. (Uganda 2000: 64% HCW Ebola infected)

VHF Specific Viral Diagnosis: Centralised or Decentralised?

Reduction in Ebola titre by RNA Extraction Buffers		
Treatment	Recovered virus	Recovery Rate (%)
Saline	8.8×10^4 pfu	58
Tripure (Roche) 1:1	None	0
NC (ABI) 1:1	1.7×10^4 pfu	11
5:1	None	0

Towner et al JID 2007

Decentralised

- Local capacity close to patient
- NAT on inactivated specimens can be done relatively safely
- Relatively straightforward to design PCR primers from the literature.
- Maximal capacity for mass exposure response (bioweapon)

Centralised:

- Very low clinical case numbers
- Need to build critical mass of experience/credibility
- TAT \leq 1 day from any major Australian city
- Capacity for hundreds of tests per day
- Access to a range of assays & techniques
- Viral culture available
- PC4 labs expensive/challenging to run and need support
- Experience and confidence can enhance safety.
- Access to control material extremely limited

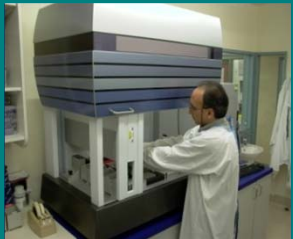
VHF Specific Viral : Centralised or Decentralised?

International Consensus is referral of specific virus diagnosis to expert centres with PC4 containment laboratories:

- USA: Send specimens to CDC or USAMRID
- UK: Send specimens to HPA (Collindale or Porton Down)
- EU: Specimens sent to European Network for Diagnostic of Imported Viral Diseases (ENVID) reference laboratory
- Canada: Send specimens to National Microbiology Laboratory (Winnipeg)

Some national guidelines (UK, EU) provide for exclusion of differential diagnoses in hospital laboratories for selected lower risk patients.

National High Security Quarantine Laboratory: Emergency Diagnostic Capacity Exercise



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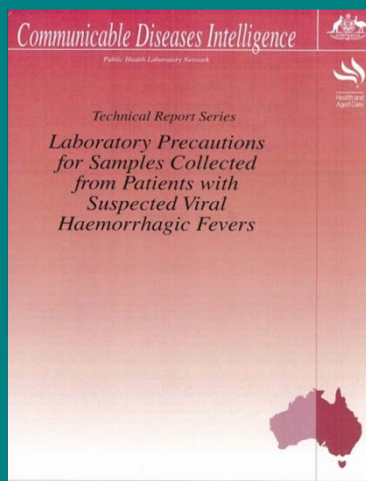
Requirements for a Physical Containment Level 4 (PC4) Laboratory



- Separate building or shell
- Sealed internal shell facilitating cleaning/fumigation
- Controlled access
- Outer/inner change room separated by shower with interlocked doors
- Separate supply/exhaust air with inward directional flow at 25Pa drop between areas
- HEPA filtered exhaust air & supply air
- Flexible film isolators vented via 2 x HEPA filters in series or,
- Positive pressure suits with life support system and back up
- Double sided autoclave
- Pass through dunk tank and/or fumigation chamber
- Liquid waste decontamination by chemical or heat
- Back-up emergency power



VHF Clinical Pathology: Decentralised



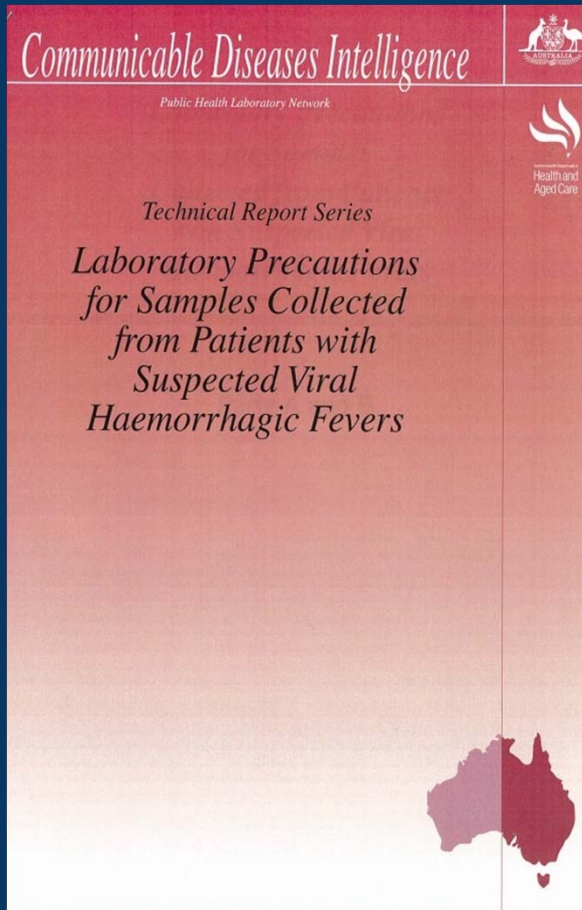
• Challenges:

- Decentralised care: hospital laboratories responsible for clinical pathology
- Infrequent cases: dedicated laboratories/equipment not feasible
- Non-specific early syndromes: potential delayed recognition of cases
- Unwell patients: may require high intensity testing
may require a wide range of tests

• Guidelines

- PHLN produced guidelines for safe testing of VHF samples
- Part A: for laboratories other than the designated State VHF isolation hospital.
- Part B: for laboratories associated with a designated isolation hospital

Laboratories Not Associated With a Designated Isolation Hospital



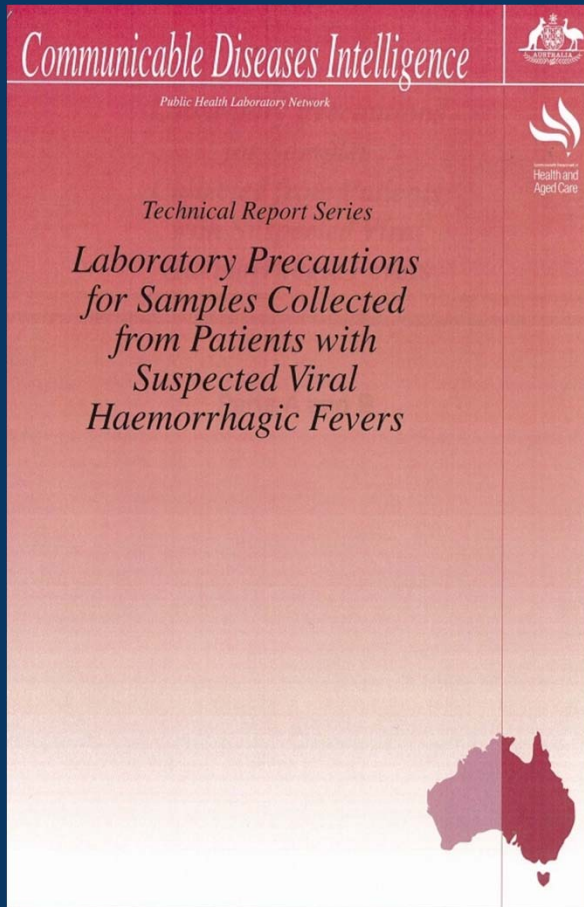
Scope of sampling and testing

- Kept to the minimum necessary for patient management
- Minimise urgent and out of hours testing
- Appropriate PPE, minimise sharps, specimen contained.

Laboratory receipt and processing

- Separate room/area with BSC class I or II
- Designated senior staff
- PPE: long sleeve gown, gloves, shoe covers, P2 mask, eyewear
- PPE disposed by incineration/hypochlorite soak after use
- Non-inactivated specimens ok in closed automated decontaminable analysers
- Specimens inactivated if possible:
 - heat 60°C x 60 min for U &E, NAT
 - heat 58°C x 60 min for serology
 - thick/thin films + histology +
 - IFA: solvent fixation
- Avoid aerosolization/sharps

Laboratories Associated With a Designated Isolation Hospital



Specimens/Scope/Transport

- Testing kept to minimum necessary for patient management
- Transport scheduled and lab advance notified
- Safe, planned transport logistics, specimen accompanied.

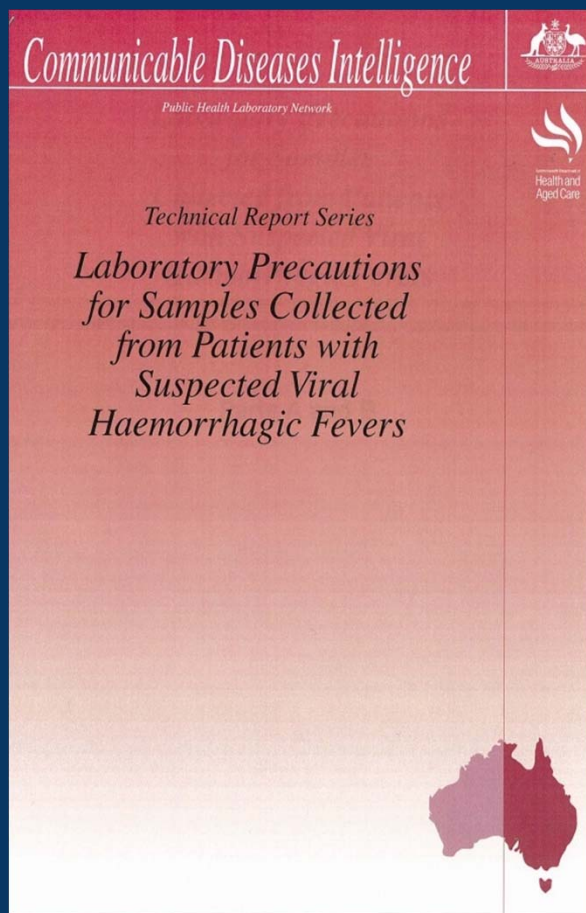
Designated Receiving Area (DRA)

- Designated and equipped room for sample processing/storage/disposal.

Testing Laboratory Areas: non-inactivated samples

- Senior staff member coordinates/liaises
- Optimum scheduling, limited access to area
- Experienced staff, PPE
- Samples opened in BSC, aerosol/splash avoided
- Cleaning decontamination as recommended.

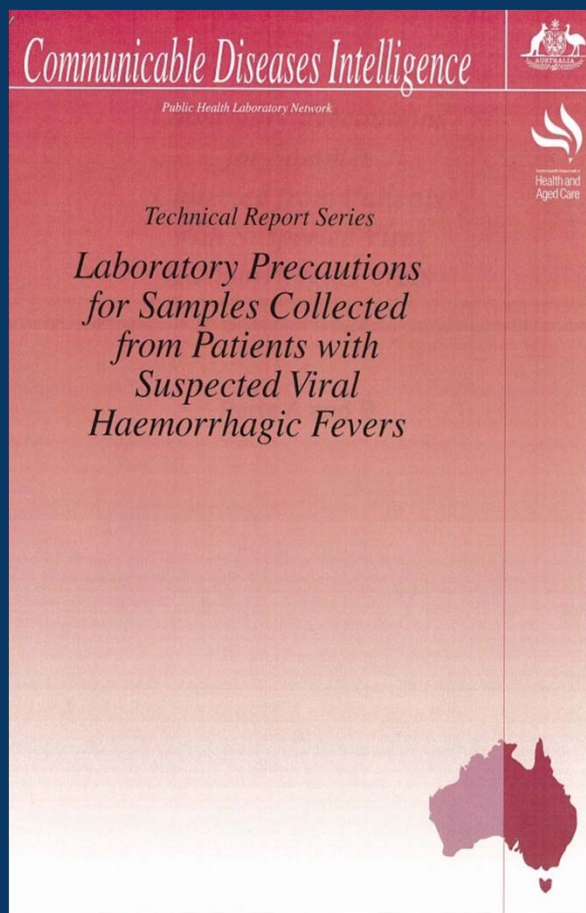
Laboratories Associated With a Designated Isolation Hospital



Designated Receiving Area – for Initial sample processing and storage/disposal

- Supervised by senior micro/virology staff member
- Experienced personnel
- Separate, sealable room with lockable door (closed + signage)
- BSC, lab sink + basin, fridge + freezer, heat block, centrifuge (sealed buckets)
- Autoclave nearby, shower/change room optional
- PPE (gloves, impervious long gowns, overalls, N-95 mask, eye protection)
- Biohazard bag for used clothing
- Sharps container
- Betadine hand-wash
- 0.5% hypochlorite wipe of bags, and immersion eyewear
- Double bag & hypochlorite wipe all waste, then autoclave

Laboratories Associated With a Designated Isolation Hospital



Specialty Areas

- Haematology: discussion with unit head before testing
 - Malaria thick/thin
 - haematocrit, blood film and differential
 - Haemoglobin
 - coagulation studies
- Blood cross match can't be done safely
- Thick & thin films prepared in the DRA (ditto blood film)
- Coulter counter and coagulation machine OK, provided tops stay on tubes, waste fluids properly disposed of, and machine decontaminated.

Laboratories Associated With a Designated Isolation Hospital

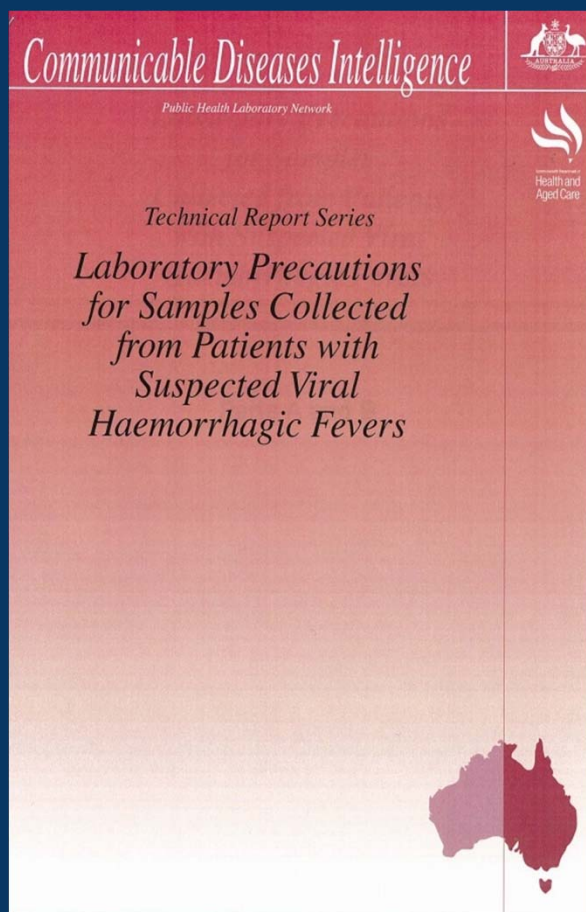
Specialty Areas

Biochemistry: discussion with unit head before testing

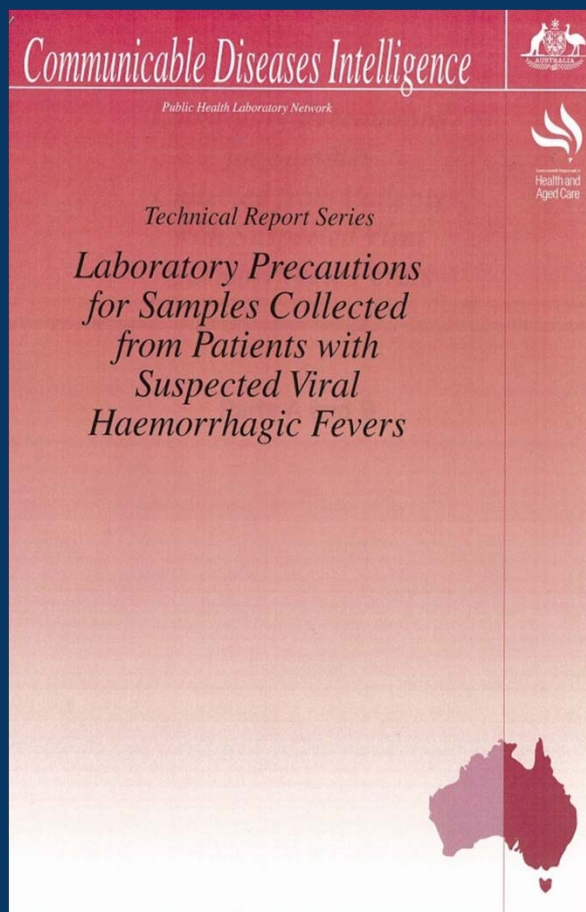
- U & E - blood gases
- LFT
- Inactivated sera wherever possible (repeat once VHF excluded)
- Non-activated sera in routine analyser as for haematology.

Bacteriology: discussion with unit head before testing

- Routine diagnostic bacteriology: CSF, blood, urine, sputum, faeces, genital, wound
- Plated with disposable item in DRA
- Seal and incubate in Co2 incubator in DRA (or jars as appropriate)
- No automated blood cultures where routine venting either:
 - Subculture to agar, or
 - Fully enclosed automated system
- Subcultures in DRA, secondary cultures in routine laboratory.



Laboratories Associated With a Designated Isolation Hospital



Specialty Areas

Virology: discussion with unit head before testing

- VHF diagnostic testing at NHSQL
- Cell Culture must not be undertaken
- IFA can be done on slides fixed in DRA
- NAT can be performed on inactivated samples (heat/lysis buffer)

Serology: discussion with unit head before testing

- Use of heat-inactivated sera wherever possible (repeat after VHF excluded)
- If non-inactivated serum testing absolutely necessary:
 - DRA processes up to wash step following serum incubation
 - Remaining steps in routine laboratory

Immunology: discussion with unit head before testing

- Heat inactivated sera used for autoantibody tests (repeat after VHF excluded)
- IFA done after slides prepared in DRA
- Use of non-inactivated samples as for serology
- NAT can be performed on inactivated samples as for virology
- Complement assays and CMI tests cannot be performed

Specimen collection, transport, storage

Collection

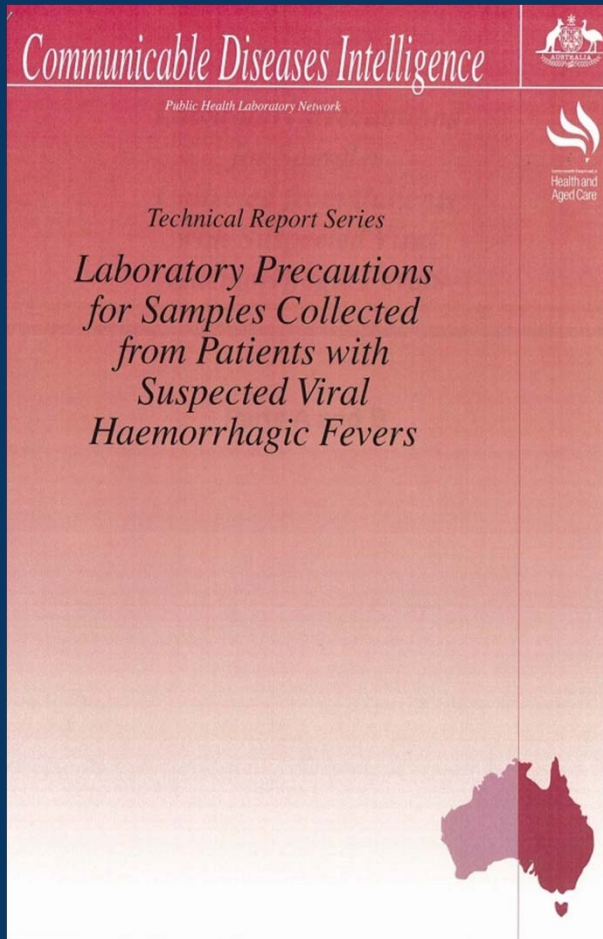
- Avoid external contamination of container during collection
- Plastic bag, within rigid outer container
- 0.5% hypochlorite wipe/spray outer container

Transport

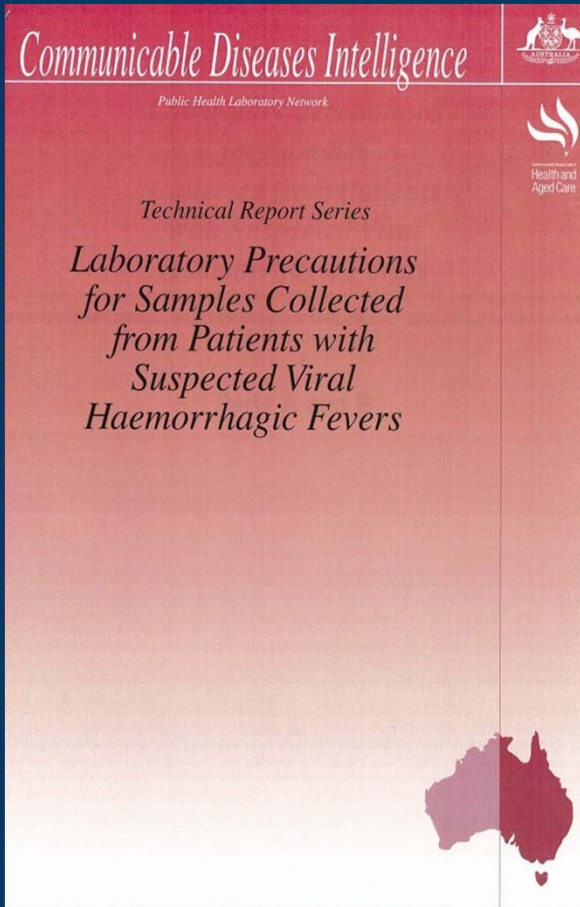
- Direct transport to DRA accompanied at all times
- No vacuum tubes

Processing

- Inactivation in DRA if possible.
- Sub-samples 0.5% external hypochlorite wipe outside and re-package
- Labelling as 'inactivated-no VHF Risk' or otherwise
- All non-inactivated samples returned to DRA for storage/disposal

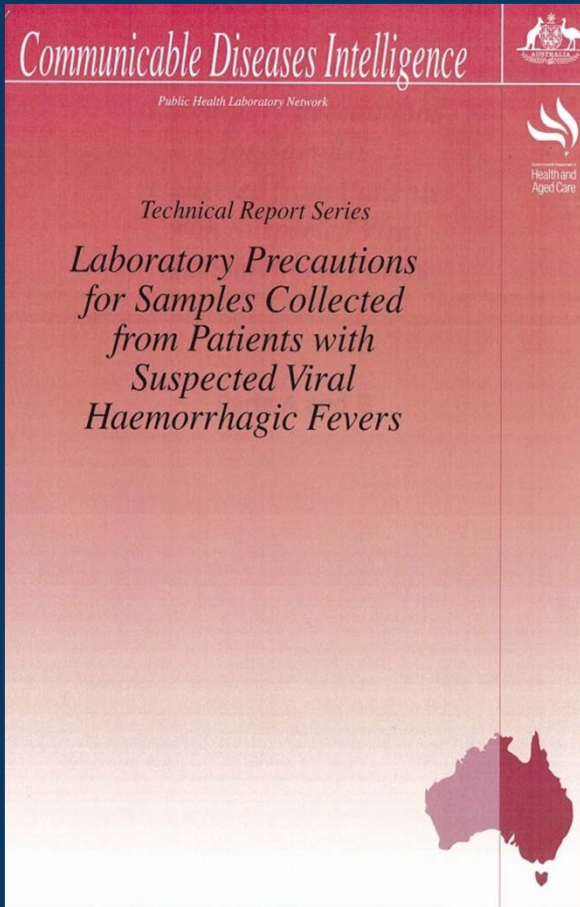


Sample Inactivation



- Heating 60^oL x 60 min of serum/body fluids
 - Suitable for U & E, bilirubin, glucose, CRP and NAT
 - Not suitable for enzymes such as ALK phos, ALT, GGT, CR, or serology
- Heating 57^o x 60 min of serum/body fluids
 - Suitable for serology
- 10ml x 10% Triton X-100/ml sample x 1 hour
- 10% buffed formalin x 15m
 - Suitable for air-dried malaria thick/thin films
- Methanol x 5min, then 10% buffered formalin x 15 min, or Methanol x 30 min, then dry heat 95^oC x 1 hr
 - Suitable for malaria thin films
- 10% buffered formalin or 2.5% glutaraldehyde fully penetrating specimen
 - Tissue for histology
- Acetone 85-100%, glutaraldehyde ≥ 1% buffered formalin x 15 min
 - Suitable for IFA slide fixation

Cleaning and Decontamination



- Abundant supplies of disinfectants needed (fresh daily)
 - 0.5% hypochlorite
 - 70% ethanol
 - 1% glutaraldehyde (note toxicity)
 - Betadine, chlorhexidine/alcohol hand washes
- BSCs cleaned after VHF sample work
 - Wipe 0.5% hypochlorite, 10 min rest, wipe off, or
 - Wipe 1% glutaraldehyde, vacate room, 10 min rest
- Centrifuge buckets/rotors autoclaved or immersed 1% glutaraldehyde x 10 min
- Automated machinery decontaminated 0.5% hypochlorite x several cycles, plus external 0.5% hypochlorite wipeover
- Manufacturer's alternative protocol ok if VHF validated.
- 20 uninfected samples, or saline equivalent through analyser before routine use
- Specimen racks double bagged and returned to DRA for autoclaving
- Spills: Cover with pad soaked in 1% hypochlorite and soak x 30 min
- Wipe with absorbent material soaked in 1% hypochlorite
- Double biohazard bag on waste for autoclaving
- Significant spill outside BSC: evacuate, close for 1 hour then clean up as above.

Conclusion



- Extremely rare syndrome caused by a group of exotic viruses
- Long incubation periods and non-specific prodrome
- Secondary transmission uncommon in developed countries
- High mortality, limited therapeutic options
- Laboratory accident potential
- RT-PCR on blood plasma the mainstay of diagnosis
- EIA or IFA serology useful adjunct in non-fatal cases
- Specific viral diagnosis centralised on PC4/BSL4 laboratories internationally
- Some decentralisation feasible in mass exposing event (bioweapon)
- Clinical pathology will be done in hospital pathology departments.
- Australia has guidelines for how to organise this.