

## Prevention of infections in an ART laboratory: a reflection on simplistic methods

C. HUYSER

*Department of Obstetrics and Gynaecology, University of Pretoria, Steve Biko Academic Hospital, Pretoria, South Africa.*

Correspondence at: carin.huysen@up.ac.za

### *Abstract*

Preventative measures combined with reactive remedial actions are generic management tools to optimize and protect an entity's core businesses. Differences between assisted reproduction technology (ART) laboratories in developing versus developed countries include restricted access to, or availability of resources, and the prevalence of pathological conditions that are endemic or common in non-industrialized regions.

The aim of this paper is to discuss the prevention of infections in an ART laboratory in a low to middle-income country, with reference to simplistic risk reduction applications to avoid the introduction and transmission of pathogens. Diagnostic and procedural phases will be examined, i.e. (i) screening for microbes during patient evaluation, and (ii-iii) prevention of environmental and procedural contamination.

Preventative action is enabled by knowledge of threats and the degree of risk involved. Awareness and understanding of the vulnerabilities in an ART system, wherein laboratory personnel operate, are invaluable assets when unforeseen equipment failure occurs or instant decisions have to be made to safeguard procedures. An inter-connective team approach to patient treatment, biosafety training and utilization of practical procedures such as semen decontamination, are fundamental tools in a laboratory's risk-reduction armoury to prevent and eliminate infectious elements.

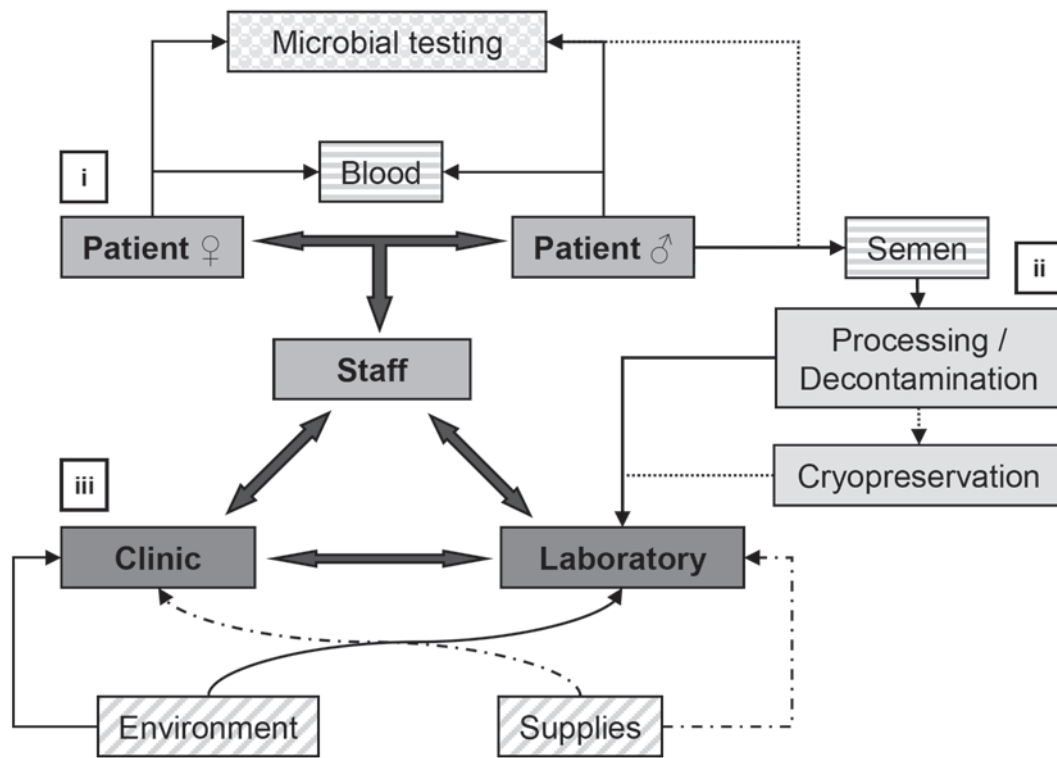
**Key words:** ART, assisted reproduction, developing countries, HIV-1, infections, laboratory, prevention.

### **Introduction**

"Any uncertainty about a future event that might threaten an organization's ability to accomplish its mission" may be labelled as a risk. Proactive prevention as opposed to reactive cure can be seen as prophylactic management to provide quality assisted reproduction technology (ART) services (Mortimer and Mortimer, 2005). Reactive actions such as timeously troubleshooting with corrective adjustments and evidence-based handling of adverse events during ART procedures are also invaluable risk management tools.

Developing countries are constrained by limited access to, or availability of resources, which will impact on the screening, diagnosis and management of infectious diseases. The 2014 West African Ebola outbreak is a current example, with numerous media reports outlining the prevailing stigma and

conspiracy theories on the origin and progress of the disease. Undoubtedly disgrace, ignorance, limited finances and resources similarly impede the extent and frequency of screening for sexually transmitted infections (STIs) prior to ART, selection of ART procedures, and choice of a private or public ART provider in low to middle-income countries (Huysen and Fourie, 2010). The aim of this paper is to deliberate the prevention of infections in a laboratory-orientated ART setting in a low to middle-income country, with reference to simplistic risk reduction applications to avoid the introduction and transmission of pathogens. Diagnostic and procedural laboratory segments during ART will be briefly discussed, as indicated in Figure 1, i.e. (i) screening for microbes during patient evaluation, and (ii-iii) technical and environmental factors including sperm preparations to reduce infectious agents.



**Fig. 1.** — Elements that influence the prevention of infections during an ART program: (i) Diagnostic phase, i.e. patient assessment and screening, and (ii-iii) the procedural phase including sperm processing, environmental and technical factors (Huyser, 2014).

### Patient screening for microbes: detection and prevention

Work-up of couples prior to ART treatment and handling of human bodily fluids (blood, follicular fluid and semen) during procedures should be generic for all patients, irrespective of the type of ART procedure (Huyser, 2014). Various viral agents such as cytomegalovirus, hepatitis B (HBV), hepatitis C (HCV), herpes simplex virus type 2, human T-lymphotrophic virus, and human immunodeficiency virus (HIV) can be transmitted through semen and vaginal secretions (Elder et al., 2005). A detailed review and guidelines of infections in the female and male partner, and infectious complications of ultrasound-guided oocyte retrieval is provided by Steyaert et al. (2000).

The practice of routine microbial semen cultures in asymptomatic couples is debatable; however patients should be routinely screened for HIV, HBV/HCV (blood plasma) as well as other predominantly prevalent STIs (in the region) and laboratory personnel notified of the test results prior to the ART attempt (Magli et al., 2008).

Sexual health screening in most developing countries where ART is offered, is probably less state-regulated with infrequent and fewer tests over time. Most ART practices in developing countries use point-of-care testing that offers overall

affordability, and a short time-span to perform, interpret and provide the results. The use of rapid STI screening tests with high sensitivity, specificity and long shelf-life presently plays a significant role in infectious disease screening and reproductive health management in resource-constrained countries (Huyser and Fourie, 2010). Technological advancement of currently available rapid tests is needed e.g. direct assessment in seminal plasma as well as blood plasma, with the ability to grade viral load levels to a certain degree.

Laboratory operating procedures and staff conduct that eliminate or reduce the transmission risk of microbes during ART procedures should be a central theme during training, especially when resources to test or monitor aspiring ART patients are lacking or infrequently provided.

### ART procedures: gametes and embryos

#### *Environmental and technical factors*

ART laboratory personnel are trained to “treat each sample as potentially infectious” and “to ensure aseptic conditions for gametes, zygotes and embryos” (Magli et al., 2008). Transmission of infections can be from health workers to patients or from patients to staff-members, other patients or the immediate environment (Junk, 2008). Safeguarding

through universal precautions and adherence to the safety procedures outlined in the WHO laboratory manual for the examination and processing of human semen (WHO, 2010) is usually provided as guidelines when in doubt. To take extraordinary preventative action implies having knowledge of threats and the degree of risk consequently involved. Awareness and understanding of the vulnerabilities in an ART system, wherein laboratory personnel operate, are invaluable assets when unforeseen equipment failure occurs or instant decisions have to be made to safeguard procedures. Sources of infection, are most commonly infected patients/staff, and less frequently the immediate environment (Junk, 2008). Simple laboratory organisational precautions include uncluttered work surfaces, phasing out of sharp-edged glass items, no mouth pipetting, hygienic working conditions (procedures using appropriate equipment and disposables), regular cleaning and decontamination of the laboratory (quality control measures), proper (hazardous) waste disposal and strict access control to different laboratory sections. On a personal level, skin breaks should be protected with waterproof dressings and non-toxic powder-free gloves should be worn at all times, together with appropriate attire (barrier precautions - clothing, masks, gowns, and goggles) (Steyaert et al., 2000; Elder et al., 2005; Junk, 2008; Magli et al., 2008). Personnel should be immunized against HBV with a baseline serum result available (Elder et al., 2005) as well as against other viral diseases for which vaccines are available (Magli et al., 2008).

Biosafety for ART procedures are classified as level 2, with supplementary precautions when processing HIV and HBV/HCV-positive samples. Specimens from patients who tested positive for blood borne viruses should be processed, cryopreserved and stored in dedicated areas using separate storage tanks with adherence to specific safety measures (Magli et al., 2008). Sero-positive patients can alternatively be batched or scheduled to allow sufficient decontamination of the laboratory (Magli et al., 2008) after contact with patient's body fluids, i.e. semen processing, follicular fluid-aspirations and embryo transfer. The significance and consequence of risk factors involved in infectious agents/conditions will impact directly on the decontamination method applied, i.e. physical cleaning, disinfection and sterilization (Junk, 2008). All procedures and manipulations that can produce aerosols or splatter should be performed in Class II biological safety cabinets (BSCs) with vertical laminar flow, using aseptic techniques and sterile disposables (Elder et al., 2005; Magli et al., 2008). Best practice is to ensure separate facilities and

equipment for semen handling and cryopreservation within an ART laboratory (Elder et al., 2005). Most ART laboratories in developing countries only have a single BSC for all laboratory material processing, a single centrifuge, stereo microscope, with open bench sperm processing. Batching and staggering of laboratory activities in the BSC according to a workable timeline can stratify and spread activities out in a safe manner. The use of a single-step embryo culture system should also minimize manipulations and exposure to unfavourable conditions outside of the incubator. In addition, microdroplet cultures under oil (Magli et al., 2008) should speedup embryo evaluation and protect the culture medium from environmental contaminants when only large upright incubators are available; and/or air filtration or a positive pressure system is *not* available. These preventative actions are especially applicable in humid tropical countries where apartments or houses are modified and adapted to function as ART units.

Cryopreservation is often an expensive necessity during ART in developing countries. An array of protocols, devices and publications on biosafety trials outlining the potential for disease transmission and pathogen survival during cryopreservation are currently available. If a cryopreservation protocol could be designed that can be universally applied, the worldwide use thereof could translate in an economical and safe system (Scholtz, 2012). According to Scholtz (2012) commercial liquid nitrogen does not pose a sufficient high risk for contamination, whereby prevention of cross-contamination in containers using closed or semi-closed vitrification devices that permits sealing as well as a high cooling rate should be sufficient to warrant sterility, good survival and development of the specimen cryopreserved.

#### *Semen processing: Decontamination procedures*

Semen samples should be handled with extreme care as a biohazard by laboratory personnel (WHO, 2010). In-house statistics have indicated the positive identification of microbes in approximately 50% of all semen samples obtained for ART procedures, with gram-negative species present in only a fraction of samples (Huyser and Fourie, 2010; Fourie et al., 2012). In addition, over 50% of all neat semen samples from HIV-1 infected males tested consistently positive for HIV-1 RNA (quantitative result) or DNA (qualitative result) over a 5-year period (Huyser and Fourie, 2010; Fourie et al., 2014) (Table I – reviewed data).

Antimicrobial treatments are prophylactic or empirically prescribed to intended ART patients

**Table I.** — HIV-1 RNA and DNA detection in neat semen and purified sperm samples from HIV-1 positive males (N = 104) in preparation for an ART cycle.

Neat Semen Samples (N = 236)				Processed Sperm Samples (N = 160)	
Negative		Positive		Negative	
DNA & RNA	DNA	RNA	DNA & RNA	DNA	RNA
46.19%	15.68%	19.91%	18.22%	98.75%	100%
N = 109	N = 37	N = 47	N = 43	N = 158	N = 160

when pathology services are not readily available to the couple. Sperm purification through discontinuous density gradient centrifugation is used, in preparation for intra-cytoplasmic sperm injection (ICSI). In this scenario seroconcordant or discordant couples are restricted to the ICSI procedure with confounding costs; in cases where ICSI is not offered at the ART Unit the couple have to add travel and accommodation costs.

An array of sperm processing methods are commercially available, from simple office-based semen preparation kits for intra-uterine insemination to density gradient centrifugation with an additional mechanical device to reduce microbe recontamination in the processed sample (ProInsert™ system, Nidacon, Sweden) (Huyser and Fourie, 2010; Fourie et al., 2014). Once the enriched sperm fraction is removed, the device can be capped and discarded as biohazardous waste. In our program, semen decontamination eliminated HIV-1 RNA (100% non-detection) and proviral DNA from 98.75% of semen samples of HIV-1 positive patients, as shown in Table I. A robust and affordable semen decontamination method is a valuable risk reduction tool to eliminate bacteria (Fourie et al., 2012) and viruses (Huyser and Fourie, 2010; Fourie et al., 2014) within a low-resource setting.

## Conclusion

The prevention of infections within any ART laboratory is a team effort which starts with the couple's reproductive health work-up to identify and avoid vertical and horizontal transmission and nosocomial transfer of pathogens during an ART cycle. Preventative action is enabled by knowledge of threats and the degree of risk involved. Herewith biosafety training and utilization of practical procedures such as semen decontamination are

fundamental tools in any laboratory's risk-reduction armoury to prevent, reduce or eliminate infectious elements.

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