



## Risk Assessment – VI-RA-016- Reverse Transcriptase Colorimetric Assay

### Scope

The Reverse Transcriptase Assay, colorimetric, takes advantage of the ability of reverse transcriptase to synthesize DNA using the hybrid poly (A) x oligo (dT)<sub>15</sub> as a template and primer. In place of radiolabeled nucleotides, digoxigenin- and biotin-labeled nucleotides in an optimized ratio are incorporated into the same DNA molecule by the RT activity. A template/primer hybrid is supplied, but the flexibility of the assay allows the use of a template of choice (*e.g.*, a viral template). The detection and quantification of the synthesized DNA as a parameter for RT activity follows a sandwich ELISA protocol: biotin-labeled DNA binds to the surface of streptavidin-coated microplate modules. In the next step, an antibody to digoxigenin, conjugated to peroxidase (anti-DIG- POD), is added and bound to the digoxigenin-labeled nucleotides. In the final step, the peroxidase substrate ABTS is added. The peroxidase enzyme catalyzes the cleavage of the substrate to produce a colored reaction product. The absorbance of the samples is determined using a microplate (ELISA) reader, and is directly correlated to the level of RT activity in the sample.

<b>Carried out by:</b>	Tiphaine Bouriez-Jones	<b>Date carried out:</b>	May 2015	<b>Review Due:</b>	May 2018
<b>Hazard (Cause and consequence)</b>	<b>Affected Groups</b>	<b>Existing controls</b>		<b>Risk</b>	<b>Further actions</b>
Infection from exposure to pathogens - Via direct contact with the pathogen (i.e. skin adsorption from splash) - Via spill of material - Via incorrect disposal of waste	Staff Students and visitors	<b>CL3 biological agents</b> Only trained users who have shown evidence of their experience to the CL3 Safety Officer will have access to the CL3 suite out of hours.  Each user is trained to adhere to the CL3 Code of Practice, they will follow the precautions involved with handling and storing pathogens.  Every user must double glove, wear a leak-resistant disposable gown and wear safety spectacles whilst working in the suites. The use of sharps is forbidden in the CL3 suites.  Out of hours workers must at least have a buddy system in place or work in pairs.		Medium	Bi-yearly checks on the BSC within CL3  Yearly checks on BSC in CL2



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		<p>Users are familiar with emergency procedures and a spill drill is implemented as a check on measures.</p> <p>A telephone available in each CL3 suite, with up-to-date list of emergency contact details next to it.</p> <p>Waste is autoclaved within the suite, samples will be packaged in tertiary container if they need to be taken outside of the CL3 suite.</p> <p>No engineer is allowed to work out of hours in the CL3 suite.</p> <p><b>CL2 biological agents</b></p> <p>Users are trained to follow good microbiological practice. They must wear blue labcoat, nitrile gloves and safety spectacles at all time whilst working in CL2.</p> <p>Procedures in case of spill or exposure policies are explained at induction and the policies are displayed in the CL2 laboratories.</p>		
Being trapped in the CL3 suite out of hours (door release mechanism no longer functioning)	Staff Students and visitors	Emergency release of the door mechanism present on each door.	Low	Yearly maintenance service contract
Entering the suites under duress	Staff Students	There is a duress code which can be entered instead of the normal code which will raise the alarm directly to Security Services without the knowledge of the persons entering the suites.	Low	Tested yearly by Facilities
Loss of containment	Staff Students and visitors	<p>An audible alarm is triggered in case of loss of containment to alert users.</p> <p>All users are familiar with the emergency procedure in case of loss of containment: securing their work, leaving the facility</p>	Low	Pressure monitored weekly, yearly



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		without delay and alerting Facility as soon as possible to resolve the issue.		maintenance contract.
Injury due to misuse or faulty equipment	Staff Students and visitors	All users are trained in the correct operation of instruments. Specialised equipment such as centrifuges and incubators are under maintenance service contract.	Low	Incubators and centrifuges serviced yearly
Exposure to chemicals (Ethanol, Industrialised Methylated Spirit, Virkon)	Staff, students and visitors	Via Inhalation: Where possible stock will only be available in solution. Virkon is available as powder due to the difficulty of dissolving and attaining the appropriate concentration, users must be careful when dispensing Virkon and always cover the lid of the stock pot. Via skin adsorption: User must wear gloves and labcoat at all time. Via instillation (eye): User must wear safety spectacles at all time. <b>See specific COSHH risk assessment for each chemical.</b>	Medium	Checks on LeV
Asphyxiation in oxygen deficient atmospheres.	Staff Students Visitors	Oxygen level sensors and air change extraction system.	High	Regular checks of oxygen sensor system and extraction system to reduce chance of failure
Over pressurisation from the large volume expansion of the liquid. If liquid nitrogen enters sample vials during storage, the vials when removed from the liquid nitrogen can become rapidly over pressurised and explode in the face of the user.	Staff Students	It is <b>imperative</b> that a face shield and safety glasses are worn when handling samples that have been stored in the cryostorage units. Storage racks are being modified to prevent the storage of samples in the liquid phase.	High	Regular checks on the integrity of the face shield. Monitoring use of face shield



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Cold burns, frostbite and hypothermia from the intense cold	Staff Students	Eye protection, glasses or face shield (dependant on splashing risk), closed shoes, lab coats and cryo gloves <b>must</b> be worn when handling liquid nitrogen.	Medium	PPE are checked on a regular basis by Facilities
Release or exposure to biological pathogens within the cryogenic facility	Staff, students and visitors	<p>Samples must be prepared in Biological Safety Cabinet and be placed in “Mr Frosty” prior to freezing in a -80 within the CL3 facility. After 24hr vials can be transferred to the cryogenic facility. The outside of the container must be sprayed with 70% IMS before it can be brought out of containment.</p> <p>Users must refer to the Biological COSHH assessment prior to starting work to understand the precautions associated with the microorganism that will be used.</p> <p>In case of accidental release, the cryogenic facility will be shut, until the room has been fully decontaminated and the BSO will decide when the facility is safe to re-open.</p>	Medium	None
Exposure to chemical present in the kit (irritant)	Staff, students and visitors	<p>Users are trained to use and store chemicals safely.</p> <p>Inhalation: Stock are in solution only – no aerosol should be produced in this assay.</p> <p>Skin adsorption: Users must wear gloves whilst working in CL2 laboratories.</p> <p>Eye instillation: users must wear safety spectacles at all time whilst working in CL2 laboratories.</p>	Low	None

It is the users responsibility to ensure what controls are needed to ensure that the health of themselves and others around them. It is imperative that you **DO NOT** start any work until you are absolutely sure of the appropriate precautions that need to be employed. If you are unsure seek advice from your line/laboratory manager or your departmental safety officer (DSO).