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Drawn Date Rev. 01	V. Grimm 25.02.2013	First Time Use of CarnoCheck® Laboratory SOP/equipment/consumables checklist	

INTRODUCTION

This guideline was written by the manufacturer of CarnoCheck® in order to facilitate the setting up of the laboratory equipment prior to the first time use of the CarnoCheck® Kit. The compliance with the present guideline should be considered to be a prerequisite for the successful application of CarnoCheck®. Furthermore please follow the instructions for use of CarnoCheck®.

Part I briefly pictures the separation of the laboratory into four separate rooms whereas Part II describes in detail equipment and consumables necessary within each laboratory room. Part II further specifies additional instructions to be carefully followed. Please proceed with the guideline for installation of the CheckScanner™ and the CheckReport™ software in order to perform your first analysis with CarnoCheck®. Part IV represents a checklist for equipments and consumables.

PART I

ROOM SEPARATION OF THE LABORATORY

In **Figure 1** the space separation of the laboratory into four separated rooms is highlighted. Every room should be solely utilized for the indicated technique in order to prevent contaminations.

Room 0: SAMPLE COLLECTION AND HOMOGENIZATION

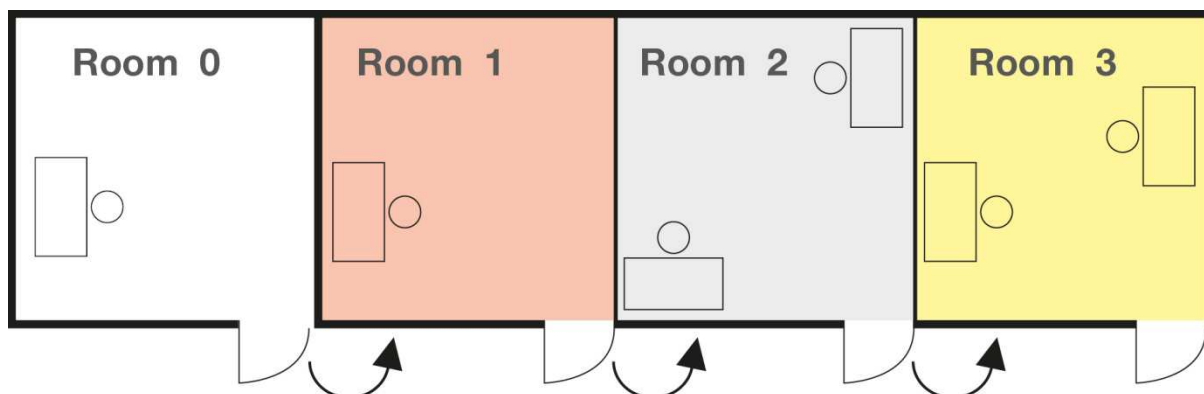
Room 1: DNA EXTRACTION


Room 2: PCR

Room 3: HYBRIDISATION

Recommendations:

- Usage of a color code for clarification of the separation as well as for avoidance of an interchange of laboratory equipment e.g. micropipettes or reaction tubes
- Change of lab coat after leaving one of the laboratory rooms
- Preferable: integration of a lock between the rooms for homogenisation, DNA extraction and the room for PCR set up



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PART II

EQUIPMENT AND CONSUMABLES

Room 0: SAMPLE COLLECTION AND HOMOGENIZATION	<p>Sample collection and homogenization has to be performed in this room. After leaving this room the lab coat has to be changed. Owing the fact that within the whole procedure of sample collection and homogenisation is a critical process step in relation to contamination, attention has to be paid to fulfill all instructions delieated in this document. For sample collection we strongly recommend the usage knives with disposable blades. As homogenizer we recommend the usage of laboratory blenders such as the Stomacher® Laboratory homgenizers or other suitable single use equipment.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td rowspan="5" style="writing-mode: vertical-rl; transform: rotate(180deg); text-align: center;">EQUIPMENT and CONSUMABLES Room 0</td> <td>precision scales</td> <td><input type="checkbox"/> available</td> <td><input type="checkbox"/> no</td> </tr> <tr> <td>homogenizer</td> <td><input type="checkbox"/> available</td> <td><input type="checkbox"/> no</td> </tr> <tr> <td>sample collection devices: knives</td> <td><input type="checkbox"/> available</td> <td><input type="checkbox"/> no</td> </tr> <tr> <td>aluminium foil plastic film bags for homogenizer</td> <td><input type="checkbox"/> available</td> <td><input type="checkbox"/> no</td> </tr> <tr> <td>Lab safety material: <ul style="list-style-type: none"> • Single use gloves • Single use lab coats Wash bottle with appropriate cleaning solution for decontamination</td> <td><input type="checkbox"/> available</td> <td><input type="checkbox"/> no</td> </tr> </table>	EQUIPMENT and CONSUMABLES Room 0	precision scales	<input type="checkbox"/> available	<input type="checkbox"/> no	homogenizer	<input type="checkbox"/> available	<input type="checkbox"/> no	sample collection devices: knives	<input type="checkbox"/> available	<input type="checkbox"/> no	aluminium foil plastic film bags for homogenizer	<input type="checkbox"/> available	<input type="checkbox"/> no	Lab safety material: <ul style="list-style-type: none"> • Single use gloves • Single use lab coats Wash bottle with appropriate cleaning solution for decontamination	<input type="checkbox"/> available	<input type="checkbox"/> no
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	Lab safety material: <ul style="list-style-type: none"> • Single use gloves • Single use lab coats Wash bottle with appropriate cleaning solution for decontamination	<input type="checkbox"/> available	<input type="checkbox"/> no														
Recommendations for SAMPLE COLLECTION AND HOMOGENIZATION	<ol style="list-style-type: none"> 1. Use a negative DNA extraction control (water or elution buffer of your extraction kit) which should be treated as a normal sample to verify that your sample batch has not been contaminated during the process. 2. As sample collection devices disposables are recommended. 3. Please carefully follow CarnoCheck® Instructions for Use. 																

**Room 1:
DNA EXTRACTION**

Standard procedure in room 1:

1. The whole DNA extraction procedure with the DNA extraction kit has to be performed in this laboratory room
2. Parts of the equipment may be replaced by comparable instruments of other manufactures. Concerning the heating block, a thermomixer is advantageous as it obviates the manual vortexing of the samples during the incubation period. The micropipettes and the rack for the reaction tubes should not be used for other purposes than DNA extraction for CarnoCheck®
3. Use either disposable lab coats for DNA extraction or change lab coat when leaving this room.

EQUIPMENT Room 1	Micropipette 0.5 -10µl	<input type="checkbox"/> available <input type="checkbox"/> no
	Micropipette 2 – 20 µl or 0.5 -10µl	<input type="checkbox"/> available <input type="checkbox"/> no
	Micropipette 20 – 200 µl	<input type="checkbox"/> available <input type="checkbox"/> no
	Micropipette 100 – 1000 µl	<input type="checkbox"/> available <input type="checkbox"/> no
	Vortex shaker	<input type="checkbox"/> available <input type="checkbox"/> no
	Thermomixer	<input type="checkbox"/> available <input type="checkbox"/> no
	Microcentrifuge for 1.5 – 2 ml reaction tubes	<input type="checkbox"/> available <input type="checkbox"/> no
	Rack for reaction tubes	<input type="checkbox"/> available <input type="checkbox"/> no
	Additional equipment (waste container, timer)	<input type="checkbox"/> available <input type="checkbox"/> no

CONSUMABLES Room 1	DNA Extraction Kit: • NucleoSpin Food	<input type="checkbox"/> available <input type="checkbox"/> no
	1.5 ml Reaction Tubes	<input type="checkbox"/> available <input type="checkbox"/> no
	Sterile micropipette filter tips for: • Micropipette 2 – 20 µl or 0,5-10 µl • Micropipette 100–1000µl	<input type="checkbox"/> available <input type="checkbox"/> no
	Ethanol puriss. p.a., ≥ 99,8%	<input type="checkbox"/> available <input type="checkbox"/> no
	Lab safety material: • Single use gloves • Single use lab coats • Wash bottle with appropriate cleaning solution for DNA-decontamination	<input type="checkbox"/> available <input type="checkbox"/> no

**Recommendations
for DNA
EXTRACTION**

1. Mark each part of equipment used in room 1 for DNA extraction by a color code in order to prevent accidental equipment exchange
2. Use only filter tips for pipetting solutions containing DNA.
3. Use disposable gloves and change them frequently.
4. Please carefully follow MN Nucleo Spin Food DNA Extraction Kit and CarnoCheck® Instructions for Use.

Room 2: PCR	<p>In Room 2 the reaction mix for the Polymerase Chain Reaction (PCR) is set up in a clean bench. The addition of the DNA extracted in room 1 to the PCR reaction mix is carried out in a separate space within room 2 . After leaving this room the lab coat has to be changed.</p> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td rowspan="12" style="writing-mode: vertical-rl; transform: rotate(180deg); text-align: center;">EQUIPMENT Room 2</td> <td>Verity™ 96-Well Thermal Cycler</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Micropipette 0.5-10 µl (for Taq addition)</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Micropipette 0.2 – 2 µl or 0.5-10 µl (fo addition of DNA eluate)</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Micropipette 5 – 50 µl (division of Mastermix)</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Micropipette 20 – 200 µl (Master-Mix set up)</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Micropipette 100 – 1000 µl</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Clean bench</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Vortex shaker</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Cooling block</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Rack for reaction tubes</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Rack for PCR tubes</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Additional laboratory equipment: waste container</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> </table> <table border="1" style="width: 100%; border-collapse: collapse;"> <tr> <td rowspan="6" style="writing-mode: vertical-rl; transform: rotate(180deg); text-align: center;">CONSUMABLES Room 2</td> <td>CarnoCheck® Kit</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>AmpliTaq® Gold DNA Polymerase (5U/µl)</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>1.5 ml Reaction Tubes</td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Reaction tubes for PCR: <ul style="list-style-type: none"> • Single 0.2 ml tubes • 8-tube PCR strips </td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Sterile micropipette filter tips for: <ul style="list-style-type: none"> • Micropipette 0.5 – 10 µl • Micropipette 100 – 1000 µl • Micropipette 5 – 50 µl </td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> <tr> <td>Lab safety material: <ul style="list-style-type: none"> • single use gloves • single use lab coats • wash bottle with appropriate cleaning solution for decontamination </td> <td><input type="checkbox"/> available <input type="checkbox"/> no</td> </tr> </table>	EQUIPMENT Room 2	Verity™ 96-Well Thermal Cycler	<input type="checkbox"/> available <input type="checkbox"/> no	Micropipette 0.5-10 µl (for Taq addition)	<input type="checkbox"/> available <input type="checkbox"/> no	Micropipette 0.2 – 2 µl or 0.5-10 µl (fo addition of DNA eluate)	<input type="checkbox"/> available <input type="checkbox"/> no	Micropipette 5 – 50 µl (division of Mastermix)	<input type="checkbox"/> available <input type="checkbox"/> no	Micropipette 20 – 200 µl (Master-Mix set up)	<input type="checkbox"/> available <input type="checkbox"/> no	Micropipette 100 – 1000 µl	<input type="checkbox"/> available <input type="checkbox"/> no	Clean bench	<input type="checkbox"/> available <input type="checkbox"/> no	Vortex shaker	<input type="checkbox"/> available <input type="checkbox"/> no	Cooling block	<input type="checkbox"/> available <input type="checkbox"/> no	Rack for reaction tubes	<input type="checkbox"/> available <input type="checkbox"/> no	Rack for PCR tubes	<input type="checkbox"/> available <input type="checkbox"/> no	Additional laboratory equipment: waste container	<input type="checkbox"/> available <input type="checkbox"/> no	CONSUMABLES Room 2	CarnoCheck® Kit	<input type="checkbox"/> available <input type="checkbox"/> no	AmpliTaq® Gold DNA Polymerase (5U/µl)	<input type="checkbox"/> available <input type="checkbox"/> no	1.5 ml Reaction Tubes	<input type="checkbox"/> available <input type="checkbox"/> no	Reaction tubes for PCR: <ul style="list-style-type: none"> • Single 0.2 ml tubes • 8-tube PCR strips 	<input type="checkbox"/> available <input type="checkbox"/> no	Sterile micropipette filter tips for: <ul style="list-style-type: none"> • Micropipette 0.5 – 10 µl • Micropipette 100 – 1000 µl • Micropipette 5 – 50 µl 	<input type="checkbox"/> available <input type="checkbox"/> no	Lab safety material: <ul style="list-style-type: none"> • single use gloves • single use lab coats • wash bottle with appropriate cleaning solution for decontamination 	<input type="checkbox"/> available <input type="checkbox"/> no
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	Lab safety material: <ul style="list-style-type: none"> • single use gloves • single use lab coats • wash bottle with appropriate cleaning solution for decontamination 	<input type="checkbox"/> available <input type="checkbox"/> no																																					
Recommendations for PCR	<ol style="list-style-type: none"> 1. Use only filter tips for pipetting solutions containing DNA. 2. In order to avoid variations due to pipetting errors use pipettes suitable for small volumes. The micropipettes should not be interchanged within the different spaces of Room 2. 3. Vortex and spin down solutions prior to transferring. 4. Please carefully follow CarnoCheck® Instructions for Use. 																																						

**Room 3:
HYBRIDISATION**

Standard procedure in room 3:


Within the third laboratory room the hybridisation reaction and washing + drying takes place. In Room 3 also the CheckScanner™ in conjunction with the CheckReport™ Software is installed for the final analysis of CarnoCheck®. There are disposable lab coats available.

EQUIPMENT Room 3	Micropipette 2 – 20 µl	<input type="checkbox"/> available <input type="checkbox"/> no
	Micropipette 10 – 100 µl	<input type="checkbox"/> available <input type="checkbox"/> no
	Micropipette 100 – 1000 µl	<input type="checkbox"/> available <input type="checkbox"/> no
	8-channel pipette 5 – 100 µl	<input type="checkbox"/> available <input type="checkbox"/> no
	Pipettor for glass and plastic pipettes	<input type="checkbox"/> available <input type="checkbox"/> no
	Rack for PCR tubes	<input type="checkbox"/> available <input type="checkbox"/> no
	Vortex shaker	<input type="checkbox"/> available <input type="checkbox"/> no
	Centrifuge for 8-tube PCR strips	<input type="checkbox"/> available <input type="checkbox"/> no
	Hybridisation Chamber with magnetic slideholder	<input type="checkbox"/> available <input type="checkbox"/> no
	Temperature controlled water bath for washing step at 50°C	<input type="checkbox"/> available <input type="checkbox"/> no
	2x oCheck® Washboxes Handle for Slideholder	<input type="checkbox"/> available <input type="checkbox"/> no
	Centrifuge for drying the CarnoCheck® chip	<input type="checkbox"/> available <input type="checkbox"/> no
	Additional laboratory equipment: waste container; timer	<input type="checkbox"/> available <input type="checkbox"/> no
CheckScanner	<input type="checkbox"/> available <input type="checkbox"/> no	

CONSUMABLES Room 3	Reaction tubes for hybridisation mix: <ul style="list-style-type: none"> • Single 0.2 ml tubes • 8-tube PCR strips 	<input type="checkbox"/> available <input type="checkbox"/> no
	Sterile micropipette filter tips for: <ul style="list-style-type: none"> • Micropipette 2 – 20 µl • Micropipette 5 – 100µl 	<input type="checkbox"/> available <input type="checkbox"/> no
	Washing of one CarnoCheck® chip: 50 ml Polypropylene Tubes	<input type="checkbox"/> available <input type="checkbox"/> no
	Plastic Pipettes for Pipettor to prepare Washing solutions	<input type="checkbox"/> available <input type="checkbox"/> no
	50 ml Polypropylene Tubes for drying of the CarnoCheck® chip by centrifugation	<input type="checkbox"/> available <input type="checkbox"/> no
	Lab safety material: <ul style="list-style-type: none"> • Single use gloves • Single use lab coats • Wash bottle with appropriate cleaning solution for decontamination 	<input type="checkbox"/> available <input type="checkbox"/> no

**Recommendations
for
HYBRIDIZATION**

1. Please carefully follow instruction for use.
2. Use only filter tips for pipetting solutions containing DNA and suitable pipettors.
3. Please carefully follow CarnoCheck® Instructions for Use.

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PART III

ADDITIONAL INSTRUCTIONS

When implementing currently used state-of-the-art techniques in molecular biology into a laboratory, these instructions must be followed to ensure both maximum safety for laboratory staff and high quality results. These instructions do not substitute the recommendations given in the CarnoCheck® Instruction for Use.

General Instructions for all rooms:

- **Lab coats** must be worn throughout the procedures and different sets of lab coats are required for each laboratory room.
- **Gloves** must be worn during each step of the analysis and must be frequently changed, especially during DNA extraction.
- **Lab cleanness:** The working place must be decontaminated with an appropriate cleaning solution
- **Reaction tubes:** Never touch the inside of a reaction tube cap.
- **Micropipette filter tips** have to be sterile.
- **Pipetting** of small amounts of liquid in the microliter range is a challenge. Therefore take care to pipette with suitable pipettors as accurately as possible. Avoid pipetting less than 2µL.
- **Labelling:** Label every reaction tube in an appropriate manner to ensure traceability.

Room 1: DNA EXTRACTION	<p>Carefully follow the instructions for use of the DNA Extraction Kit and CarnoCheck®.</p> <p>In addition:</p> <ul style="list-style-type: none"> • Adjust the temperature of the heating block before the beginning of the DNA extraction procedure. • The presence of ethanol may inhibit the PCR reaction afterwards therefore the membrane of the Spin Column has to be dried completely prior to the addition of the final DNA elution buffer.
Room 2: PCR	<p>Carefully follow the protocol for PCR set-up described in detail in CarnoCheck® Instructions for Use.</p> <p>In addition:</p> <ul style="list-style-type: none"> • Work under the clean bench while preparing the PCR Reaction Mix • Put on the UV lamp of the clean bench half an hour before you start the PCR set-up. • The <i>Taq</i>-Polymerase has always to be stored at -20°C. • Keeping the enzyme at room temperature or even on ice could lead to degradation. Therefore, try to keep the time the enzyme is not stored at -20°C as short as possible. Optimally, use a cooling block while pipetting the <i>Taq</i>-Polymerase. • Do not use less amount of the <i>Taq</i>-Polymerase than recommended in the CarnoCheck® Instructions for Use, otherwise the amplification reaction cannot take place efficiently. • Briefly vortex the ingredients for the reaction mix prior and after pipetting.
Room 3: HYBRIDISATION	<p>Carefully follow the different steps of hybridisation and washing of the CarnoCheck® chip described in detail in the CarnoCheck® Instructions for Use.</p> <p>In addition:</p> <ul style="list-style-type: none"> • Briefly vortex the hybridisation buffer prior and after adding the PCR product. • Airbubbles should be avoided when putting the hybridisation mix on the CarnoCheck® chip. • It is strongly recommended to use an 8-channel multipipette and PCR strips. If more than one CarnoCheck® chip is processed the utilization of an 8-channel multipipette is obligatory. • The surface of the CarnoCheck® chip has to be covered with liquid during all washing steps. • The CarnoCheck® chip has to be dried completely prior to the analysis with the CheckScanner™ and the corresponding CheckReport™ Software.