Instruction Manual

ClinRep® HPLC Complete Kit

Coenzyme Q10 in Whole Blood / Plasma / Serum

REF  31000

IVD  For in vitro diagnostic use

IVDD, 98/79/EC
This product meets all applicable provisions of the directive 98/79/EC on in vitro diagnostic medical devices. The EC declaration of conformity is delivered immediately on request.
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1. COMPONENTS OF THE COMPLETE KIT AND ACCESSORIES

<table>
<thead>
<tr>
<th>Order No.</th>
<th>Description</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>31000</td>
<td><strong>ClinRep® Complete Kit for Coenzyme Q10 in Whole Blood / Plasma / Serum</strong></td>
<td>1 pce.</td>
</tr>
<tr>
<td></td>
<td>for 100 assays</td>
<td></td>
</tr>
</tbody>
</table>

**Contents:**
- Mobile Phase: 1 x 31010
- IS Internal Standard: 1 x 31012
- Plasma Calibrator, lyophil.: 1 x 31013
- Test Solution: 1 x 31014
- Sample Preparation Vials: 1 x 31020
- P Precipitant: 1 x 31021
- O Oxidation Reagent: 1 x 31022
- Manual
- Quick Reference

**Components:**
- 31010 Mobile Phase: 1000 ml
- 31012 IS Internal Standard: 40 ml
- 31013 Plasma Calibrator, lyophil.: 5 x 2 ml
- 31014 Test Solution: 3 ml
- 31020 Sample Preparation Vials: 100 pcs.
- 31021 P Precipitant: 50 ml
- 31022 O Oxidation Reagent: 5 ml

**Start Accessories:**
- 31030 Analytical Column with test chromatogram: 1 pce.
- 31032 Guard Column Holder incl. 1 Guard Column: 1 pce.

**Accessory:**
- 31033 Guard Column: 5 pcs.
- FK5810 PEEK-tubing (connection of prefilter and column): 1 pce.
- FK5820 Stainless steel prefilter holder: 1 pce.
- FK5821 Replacement filter for stainless steel prefilter holder: 10 pcs.

**ClinChek® Controls:**
- 31080 Plasma Control, lyophil., Level I: 10 x 2 ml
- 31081 Plasma Control, lyophil., Level II: 10 x 2 ml
- 31082 Plasma Control, lyophil., Level I, II: 2 x 5 x 2 ml
2. INSTRUMENT CONFIGURATION

The following instruments are required for the HPLC determination of Coenzyme Q10:

- an isocratic pump
- an injection valve or an autosampler
- a column heater (30 °C)
- an UV detector (275 nm)
- an integrator or a computer with HPLC software
- a degasser (optional)
- a pulse damper (optional)
3. INTRODUCTION

Coenzyme Q10 (CoQ10) is the predominant form of ubichinone in man and plays a fundamental role for the cellular energy production within the respiratory chain. There, CoQ10 exists as a redox pair “ubichinol (UQH₂) / ubichinone (UQ)” and enables the transfer of electrons and protons on oxygen, building up ATP as a biochemical energy equivalent. Oxidation and reduction, respectively, is performed in a two-step reaction and under formation of a stable radical intermediate (see scheme 1).

Besides its primary function as an electron carrier, CoQ10 plays an important role for the antioxidative defense mechanism of the cell. The reduced form of CoQ10 (UQH₂) is an excellent antioxidant and, due to the low reactivity of the UQH* radical, an efficient scavenger to other radicals.

In this regard, the protection from so called reactive oxygen species (ROS) is of special importance [1, 2]. These compounds are responsible for the radical peroxidation of cellular lipids, a process that causes severe cell damages. As a radical chain reaction, the lipid peroxidation can be stopped by chain breaking agents, such as UQH₂ und UQH* (see scheme 2). UQ, in turn, is regenerated to UQH₂ within cell metabolism.

As a consequence, a generalised lack of CoQ10 (i.e. UQH₂ plus UQ) may result in an insufficient elimination of cell damaging ROS. The exposure towards these species is termed as “oxidative stress” and is discussed to be among the possible causes for the pathogenesis of numerous diseases, such as diabetes [3], atherosclerosis [4], Alzheimer’s disease [5], Parkinson syndrome [6], and cancer [7]. Disturbances in energy metabolism (respiratory chain, see above), caused by a lack of CoQ10, additionally contribute to the pathogenesis of those diseases.
CoQ10 can both be taken up with the diet and synthesised by the organism. For this reason, diet-related deficiencies of CoQ10 are not known in healthy persons.

The CoQ10 status is altered in a number of diseases, of which the decrease in cardiomyopathies and degenerative muscle diseases are the most studied. There, a supplementation with CoQ10 should be applied for therapy [8].

A lack of CoQ10 can arise in case of treatment with so-called statins. Those are drugs, which are used to lower the cholesterol level in patients with coronary heart disease and cardiomyopathy. For these patients, a monitoring of the CoQ10 status is recommended during statin administration [1].

This ClinRep® Complete Kit allows for a reliable determination of total CoQ10 as ubichinone (UQ) from plasma, serum, and whole blood. The test features simple sample preparation and quick analysis. For quality assurance a test solution as well as calibration and control materials are available.

For the determination of the antioxidative status and the assessment of the oxidative stress further parameters should be used for diagnosis:

- **Vitamin E, Vitamin C, β-Carotene*, Glutathione*: CoQ10 represents a “master-antioxidant” [9] that regenerates tocopheryl radicals (vitamin E) and semidehydroascorbate (vitamin C). Additionally, the carotenoid status (e.g. β-carotene) as well as the status of glutathione should be taken into consideration [8].

- **Malondialdehyde:** Malondialdehyde (MDA) is the main degradation product of radical lipid peroxidation. Thus, the MDA status directly reflects the cell damage by oxidative stress.

Appropriate test kits are available by RECIPE (*in preparation).
4. GENERAL DESCRIPTION OF THE METHOD

The present ClinRep® HPLC Complete Kit is intended for the determination of coenzyme Q10 in whole blood, plasma, and serum. The samples are prepared within a short sample preparation and are injected into the HPLC system subsequently. At this, the sample components are chromatographically separated and detected by the UV detector.

For sample preparation a defined amount of a precipitation reagent (precipitant) is put into a sample preparation vial (included in the kit, order no. 31020). Afterwards, an aliquote of the sample, followed by an oxidation reagent, is added. By use of the oxidation reagent, the reduced form of coenzyme Q10 (UQH₂) is transformed into its oxidised form (UQ). The sample then is mixed shortly on a vortex-mixer and an internal standard is added subsequently. After this, the sample is mixed vigorously for 10 min in order to ensure a quantitative precipitation of the sample matrix. The precipitate obtained is separated by centrifugation. After centrifugation the sample shows a clear supernatant, that consists of two liquid phases. An aliquote of the upper phase is used for HPLC analysis.

The calculation of the measurements is performed by using the internal standard-method via peak areas. For quality control plasma controls in different ranges of concentration are available.
5. DETERMINATION OF COENZYME Q10 IN WHOLE BLOOD, PLASMA, SERUM

5.1 Operating the HPLC system

5.1.1 Passivation of the HPLC system

If you run different applications on your HPLC system, we recommend to regularly clean the system by passivation. Otherwise problems like ghostpeaks (e.g. contaminated injection system) and/or baseline problems (e.g. contaminated detector cell) may occur. It is important that all fluidic components of the HPLC system are passivated, with exception of the Analytical Column. For passivation of the system follow the instructions below:

- Connect pump, injection system, column heater, detector and all capillaries with exception of both columns.
- Put the outlet-capillary into a safe waste container.
- Flush the system for 15 min at a flow of 1.5 ml/min with HPLC water.
- Then pump 2-propanol through the system for 10 min
- and afterwards HPLC water for 15 min.
- Flush the system for 30 min with half concentrated nitric acid (1 volume each of concentrated nitric acid (65 %) and HPLC water).
- Afterwards purge the system with HPLC water until the pH of the waste solution is neutral. Change the water in the eluent container several times to be sure that the nitric acid will be washed out of the frit.
- Finally equilibrate the system for about 15 min with the Mobile Phase at a flow rate of 1.0 ml/min.

5.1.2 Connection of the columns and the detector

- Set the temperature of the column heater to 30 °C.
- Prior to connecting the ClinRep® Guard Column and Analytical Column, briefly equilibrate the HPLC system (pump feed, pump, injection system, column heater, capillaries, detector) with Mobile Phase; this should be done by pumping the Mobile Phase through the system for 15 min at a flow rate of 1.0 ml/min.
- Switch off the pump and install both columns in the column heater, between injection system and detector. Connect the Analytical Column in flow direction and
- allow approximately 20 ml of the Mobile Phase to flow through the columns. Therefore start with a flow of 0.2 ml/min and increase slowly to the intended value of the chromatographic separation, in this application 1.4 ml/min.
- Connect a 0.15 mm I.D. capillary to the outlet of the Analytical Column and the other end to the detector inlet.
- Wait until no more air bubbles exit from the detector outlet.
- Afterwards connect a 0.50 mm I.D. capillary to the detector outlet.
- We recommend to continue circulating the Mobile Phase for further 30 min prior to injecting the first sample.
5.1.3 HPLC conditions

**Mobile Phase:** Make sure the bottle is closed well. Otherwise components of the Mobile Phase could evaporate, which alters the retention times.

**Pump:**
- Flow rate: 1.4 ml/min
- Recycling: The Mobile Phase is circulated through the system for 100 analyses. After 100 analyses a new bottle of Mobile Phase has to be used.

**Autosampler:**
- Injection volume: 30 µl
- Injection interval: 14 min
- Washing solution: The Mobile Phase has to be used as washing solution for the autosampler.

**Analytical Column:** Analyses are performed at 30 °C (column heater). The backpressure of the column should not exceed 200 bar.

**Guard Column:** The Guard Column serves for the protection of the Analytical Column and must be renewed at a pressure increase of > 30 bar. At any rate, the Guard Column must be renewed every 100 injections at the latest.

**UV-Detector:** Set the UV-detector to 275 nm. "Sensitivity" or "Range" has to be set appropriately considering on the quality of the detector used.

**Evaluation unit:** Integration stop has to be set at 14 min.

**General:** Retention times:
- CoQ10: approx. 7.3 min
- Internal Standard: approx. 11.5 min

Please note:
Depending on the HPLC system used differences to the indicated retention times may be observed. These differences do not influence the efficiency of the analytical procedure, however they must be considered with regard to the settings for autosampler and evaluation unit. A shifting of retention times may also occur due to the aging of the column or its improper use.
5.1.4 Switching off the system

Should the system not be required for use within several days, the Mobile Phase may be left circulating at a reduced rate (0.2 ml/min). For longer periods (i.e. longer than ~ 1 week), the Guard Column and the Analytical Column should be disconnected and closed tightly. The columns can be stored in Mobile Phase. The HPLC system should then be flushed at first with 50 ml of HPLC water followed by 50 ml of water/methanol (1:1).

The UV detector should either be switched to "stand-by" or switched off, in order to preserve the lamp.

5.2 Sample preparation

5.2.1 Collecting and storing of patient samples

The determination of CoQ10 can be performed from whole blood, plasma, and serum. Stored at – 20 °C the samples are stable for up to 3 months, at 4 °C for up to 3 days and at room temperature for up to 24 hours. The samples have to be stored in the dark.

5.2.2 Reconstitution of the lyophilised Plasma Calibrator

Add exactly 2.0 ml HPLC-water to the vial and leave it standing for approximately 15 min, gently swaying it from time to time. When all material is dissolved, the solution is ready to use. The concentration of the analytes is indicated on the data sheet.

5.2.3 Precipitation

Pipette 500 µl Precipitant P into a sample preparation vial (included within the kit, order no. 31020). Afterwards add at first 400 µl sample (calibrator, control, patient) and subsequently 50 µl Oxidation Reagent O. Afterwards mix the sample for 5 sec on a vortex mixer. Pipette 400 µl Internal Standard IS (cooled at ~ – 20 °C, see below) into the vial and subsequently mix the sample vigorously for 10 min on a vortex mixer. After this, centrifuge the sample for 5 min at 10000 x g.

After centrifugation the sample shows a supernatant, which consists of two liquid phases. For HPLC analysis, the upper liquid phase of the supernatant must be used.

Please note: The Internal Standard IS should be used deeply cooled at temperatures around – 20 °C (= storage temperature of IS). For this purpose, the Internal Standard should not be withdrawn from the refrigerator until immediate use.

5.2.4 HPLC analysis

Inject 30 µl of the upper phase of the supernatant, obtained by centrifugation (see section 5.2.3), into the HPLC system.

Please note: The supernatant consists of two liquid phases. For HPLC analysis, the upper phase must be used.
5.2.5 Stability of the prepared samples

Being protected against light and stored at room temperature, the stability of the prepared samples is 24 hours. Stored at 4 °C (refrigerator) the stability is 3 days and stored at –20 °C it is 3 months.

5.3 Flow diagram

Precipitation:

- Pipette 500 µl Precipitant P into a sample preparation vial (order no. 31020)

↓

1.) Add 400 µl sample
2.) Add 50 µl Oxidation Reagent O

↓ mix for 5 sec (vortex mixer)

Add 400 µl Internal Standard IS (deeply cooled*)

↓ mix vigorously for 10 min (vortex mixer)

centrifuge (5 min at 10000 x g)

HPLC analysis:

Inject 30 µl of the upper Phase** of the supernatant

Please note: * The Internal Standard IS should be used deeply cooled at temperatures around –20 °C (= storage temperature of IS), see section 5.2.3.

** The supernatant consists of two liquid phases (see section 5.2.4). For HPLC analysis, the upper phase must be used.

5.4 Test run

Using the Test Solution (order no. 31014), a test run should be carried out in order to check the separation performance of the system, prior to analysis.

Repeatedly inject 30 µl of the Test Solution until two consecutive chromatograms are identical with respect to retention times and peak resolution (see chromatograms in section 5.8).

Now check the integration parameters (e.g. run time, peak identification, marks for peak start and end). Correct the parameters, if necessary, and inject the Test Solution once again for verification.
5.5 Calibration run

The lyophilised Plasma Calibrator has to be prepared as described in sections 5.2.2 to 5.2.4.

Inject the Plasma Calibrator several times; this enables a singlepoint-calibration with averaging. When carrying out large series of analyses, we recommend injecting a calibrator every tenth sample as well as at the end of the series; this allows checking the HPLC conditions and, should for example retention times have been shifted, correction is possible without having to repeat the sample analyses.

5.6 Accuracy control

For quality control of the analytical determinations we recommend ClinChek® Plasma Controls. These controls are available for two different ranges of concentration (order nos. 31080 - 31082, see section 1).

5.7 Calculation

5.7.1 General

Ensure that each chromatogram corresponds to the appropriate sample and mark the chromatograms (only in case of integrators; unnecessary with computers). Compare each chromatogram with that of the Plasma Calibrator and check whether the peaks representing the analytes correlate in respect to the retention time. We recommend integrating also unknown peaks, as a false identification may occur due to a shift of retention time caused by temperature fluctuation. If calibrators have been run between samples, you can recalibrate without having to repeat the analysis.

Calculation of unknown samples has to be done using the internal standard method via peak areas. Alternatively the peak heights may be used.

According to the internal standard method each sample is spiked with a so-called "internal standard" prior to the sample preparation. The internal standard is similar to the analytes in terms of behaviour during sample preparation and chromatography. Any losses during the sample preparation hence can be determined by calculating the recovery. Extrapolation to 100 % recovery allows to establish the concentration of the unknown substances in the sample.

The Plasma Calibrator supplied with the kit is intended to be used for the calibration of the system. It has to be prepared like a patient sample.

The Test Solution supplied with the kit is intended to be used for the qualitative check-up of the HPLC system (integration parameters, retention times, peak separation and shape, etc.). The Test Solution is matrix-free and therefore can be injected directly into the HPLC system.
5.7.2 **Automatic method (integrator/computer)**

When using the internal standard method, the concentrations of the unknown substances are automatically calculated by the calculation unit on the basis of their recovery once a few parameters have been programmed (see section 5.7.1). Actual concentrations of the analytes in µg/l are provided without having to perform additional calculations.

5.7.3 **Manual method**

*a.) Calculation of the recovery:*

\[
REC = \frac{\text{area IS, sample}}{\text{area IS, cal.}}
\]

*b.) Calculation of the analyte concentration:*

\[
\text{conc. analyte, sample (µg/l)} = \frac{\text{area analyte, sample} \times \text{conc. analyte, cal. (µg/l)}}{\text{area analyte, cal.} \times \text{REC}}
\]

**Conversion to molarity:**

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Molecular Weight</th>
<th>Conversion</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoQ10 (Ubichinone)</td>
<td>863.3</td>
<td>µg/l x 1.16 = nmol/l</td>
</tr>
</tbody>
</table>
5.8 Chromatograms

**Chromatogram of the Test Solution (order no. 31014):**

- Internal Standard IS: 7.47 min
- Coenzyme Q10: 11.75 min

**Chromatogram of the prepared plasma calibrator (order no. 31013):**

- Internal Standard IS: 7.31 min
- Coenzyme Q10: 11.49 min
6. TEST DATA

Linearity: For the determination of linearity native serum was used. The serum was spiked with a defined amount of CoQ10 and diluted, respectively. The following range of linearity was obtained:

<table>
<thead>
<tr>
<th>Coenzyme Q10</th>
<th>Linearity [µg/l]</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>38.9 - 20000</td>
</tr>
</tbody>
</table>

Recovery: The recovery was determined by use of native serum that was spiked with Internal Standard IS and CoQ10 in three different concentrations. The samples subsequently were assayed by 5-fold determination. Related to matrix free standard solutions, that were directly injected, mean recovery rates between 94 – 108 % were obtained.

Detection limit: The lower detection limit is 19.5 µg/l.

Determination limit: The lower determination limit is 38.9 µg/l.

Precision: For the determination of the intra- and interassay precision three samples with the following concentrations were used:

<table>
<thead>
<tr>
<th>concentration [µg/l]</th>
<th>sample 1</th>
<th>sample 2</th>
<th>sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoQ10</td>
<td>545</td>
<td>690</td>
<td>1300</td>
</tr>
</tbody>
</table>

For the determination of the intraassay precision the samples were measured in 5 analytical series, each by 5-fold determination (n = 25; n: number of values per sample). As a result, the following coefficients of variation (CV) were obtained:

<table>
<thead>
<tr>
<th>Intraassay precision (CV) [%]</th>
<th>sample 1</th>
<th>sample 2</th>
<th>sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoQ10</td>
<td>2.90</td>
<td>5.18</td>
<td>2.18</td>
</tr>
</tbody>
</table>

The interassay precision was determined from 8 analytical series, each by double determination (n = 16; n: number of values per sample). Hereby, the following coefficients of variation (CV) were obtained:

<table>
<thead>
<tr>
<th>Interassay precision (CV) [%]</th>
<th>sample 1</th>
<th>sample 2</th>
<th>sample 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CoQ10</td>
<td>4.48</td>
<td>6.66</td>
<td>3.71</td>
</tr>
</tbody>
</table>
Reference ranges: **Determination from heparine plasma and serum:**

<p>| | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>“insufficient”</td>
<td>&lt; 0.4 mg/l</td>
</tr>
<tr>
<td>“adequate”</td>
<td>0.4 – 1.6 mg/l</td>
</tr>
<tr>
<td>“increased”</td>
<td>1.6 mg/l</td>
</tr>
</tbody>
</table>

According to:

Please note: The reference ranges should be established by each laboratory under standard conditions and taking into account any special population characteristics of the area. However, the values indicated may be used as guideline until the normal ranges have been established.
7. **TROUBLESHOOTING**

<table>
<thead>
<tr>
<th>PROBLEM</th>
<th>POSSIBLE CAUSE</th>
<th>SUGGESTED SOLUTION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pump error (pressure fluctuation)</td>
<td>Air in the pump</td>
<td>Open purge valve, suck off mobile phase and pump through system at maximum flow rate</td>
</tr>
<tr>
<td>Defect pump head valve</td>
<td></td>
<td>Replace</td>
</tr>
<tr>
<td>Flow rate not constant</td>
<td></td>
<td>- Check pump for constant pressure and flow rate</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Check pump for leakage</td>
</tr>
<tr>
<td>Spikes on the baseline</td>
<td>Air bubbles in the detector cell</td>
<td>Increase the pressure in the detector cell by briefly closing off the outlet tube</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(Caution: consider maximum cell-pressure) or disconnect the column and flush the</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cell with mobile phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Air bubbles in the mobile phase</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interference from mains</td>
</tr>
<tr>
<td>Baseline drift</td>
<td>System not yet in equilibrium</td>
<td>Pump mobile phase through the system for a longer period of time</td>
</tr>
<tr>
<td></td>
<td>Temperature drift</td>
<td>Check column heater</td>
</tr>
<tr>
<td></td>
<td>Mobile phase contaminated</td>
<td>Replace mobile phase</td>
</tr>
<tr>
<td>Noisy baseline</td>
<td>Detector cell contaminated</td>
<td>Disconnect column and flush detector cell</td>
</tr>
<tr>
<td></td>
<td>Mobile phase contaminated</td>
<td>Replace mobile phase</td>
</tr>
<tr>
<td></td>
<td>Column contaminated</td>
<td>Replace column</td>
</tr>
<tr>
<td>Peaksplitting</td>
<td>Column packing defect</td>
<td>Replace column</td>
</tr>
<tr>
<td></td>
<td>Defect injection valve</td>
<td>- Disassemble and clean injection system or call service</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- If Rheodyne system used, replace Tefzel rotorseal with Vespel rotorseal</td>
</tr>
<tr>
<td></td>
<td>Column at end of useful life</td>
<td>Replace column</td>
</tr>
<tr>
<td></td>
<td>Dead volume</td>
<td>Check system</td>
</tr>
<tr>
<td>PROBLEM</td>
<td>POSSIBLE CAUSE</td>
<td>SUGGESTED SOLUTION</td>
</tr>
<tr>
<td>-------------------------------------</td>
<td>-----------------------------------------------------</td>
<td>-----------------------------------------------------------</td>
</tr>
<tr>
<td>Poor recovery</td>
<td>Internal standard partially degraded</td>
<td>Compare internal standard with test solution</td>
</tr>
<tr>
<td></td>
<td>Pipettes out of adjustment or defect</td>
<td>Check pipettes</td>
</tr>
<tr>
<td></td>
<td>Injection volume too low</td>
<td>Check injection system</td>
</tr>
<tr>
<td></td>
<td>Incorrect sample preparation</td>
<td>Follow sample preparation instruction strictly</td>
</tr>
<tr>
<td>Interfering peaks in chromatogram</td>
<td>Injection system contaminated</td>
<td>- Rinse injection system with water followed by isopropanol</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- In case of automatic injectors: clean needle</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- check external rinsing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- replace needle-washing frits if necessary</td>
</tr>
<tr>
<td></td>
<td>Peaks originate from degradation products of old samples or standards</td>
<td>Use only fresh or properly stored samples</td>
</tr>
<tr>
<td></td>
<td>Rheodyne injection system</td>
<td>Replace Tefzel rotorseal with Vespel rotorseal</td>
</tr>
<tr>
<td>High backpressure</td>
<td>Accumulation of particles in the guard column / column</td>
<td>Replace guard column / column</td>
</tr>
<tr>
<td>Retention times changed</td>
<td>Column temperature fluctuates</td>
<td>Check column heater</td>
</tr>
<tr>
<td></td>
<td>Leak in system</td>
<td>Locate and eliminate</td>
</tr>
<tr>
<td>Significant decrease of detector sensitivity</td>
<td>Energy of detector-lamp too low</td>
<td>Replace detector-lamp</td>
</tr>
</tbody>
</table>
8. REFERENCES


