Carryover, as referred to in this document, is defined as sample to sample carryover that causes poor immunoassay performance. Perform the Carryover Check if sample to sample carryover is suspected as a cause of poor assay performance in a well-characterized immunoassay in Gyrolab.

The purpose of the check is to verify the presence of sample to sample carryover in order to indicate what further actions could be needed to optimize immunoassay performance e.g. optimizing use of Rexxip buffers or using a Gyrolab method that includes additional needle washing.

The check is designed to aspirate blanks, followed by the highest concentration of sample in the standard series and, finally, blanks using all sample needles in triplicates. The check also includes a standard series.

## Prerequisites

- One Gyrolab Bioaffy CD containing at least 12 unused segments.
- Standard series samples, reagents, Gyrolab Bioaffy CD, Rexxip buffer, needle wash solution and Gyrolab method specific for the immunoassay in which carryover is suspected.

## Preparation

- Perform the maintenance method "Needle desorb" before running the Carryover Check. Refer to the Gyrolab User Guide and Instrument Guide for details.
- Verify the needle configuration i.e. syringe pump 1-4 used for sample transfer and syringe pump 5 for reagent transfer according to the Gyrolab User Guide.

## Perform Carryover Check using Gyrolab Control up to version 7.2

- 1. Perform "Needle desorb" before running the Carryover Check.
- 2. Design a wizard run, using the method specific for the immunoassay, with a standard series, blank and 7 standard points, in triplicate.
- 3. Generate a sample list template with 24 unknowns in triplicate.
- 4. Open the generated sample list and edit the MP well positions according to below:

٠	Blank and standard points	B1-B8
٠	Unknown samples #1-8 (corresponds to blanks before)	A1-A8
٠	Unknown samples #9-16 (corresponds to highest std sample)	C1-C8
٠	Unknown samples #17-24 (corresponds to blanks after)	D1-D8

- 5. Prepare Carryover Check:
  - a) Dilute samples in the matrix and Rexxip buffer normally used.
  - b) Prepare the standard series, one blank and 7 standard series samples in different concentrations and place them in the microplate according to Table 1.
    - Use the highest standard sample concentration in the standard series for the immunoassay as the high concentration in the check.
    - The blank should contain the same matrix and Rexxip buffer as in the standard series.

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ID	Sample identity	MP well
U	Sample identity	position
	Standard A_Blank	B1
	Standard A_1	B2
	Standard A_2	B3
	Standard A_3	B4
	Standard A_4	B5
	Standard A_5	B6
	Standard A_6	B7
	Standard A_7	B8
#1	Blank before needle 1	A1
#2	Blank before needle 3	A2
#3	Blank before needle 5	A3
#4	Blank before needle 7	A4
#5	Blank before needle 2	A5
#6	Blank before needle 4	A6
#7	Blank before needle 6	A7
#8	Blank before needle 8	A8
#9	Highest std needle 1	C1
#10	Highest std needle 3	C2
#11	Highest std needle 5	C3
#12	Highest std needle 7	C4
#13	Highest std needle 2	C5
#14	Highest std needle 4	C6
#15	Highest std needle 6	C7
#16	Highest std needle 8	C8
#17	Blank after needle 1	D1
#18	Blank after needle 3	D2
#19	Blank after needle 5	D3
#20	Blank after needle 7	D4
#21	Blank after needle 2	D5
#22	Blank after needle 4	D6
#23	Blank after needle 6	D7
#24	Blank after needle 8	D8

### Table 1 The samples placement in MP

- 6. Run the Carryover Check. Use the edited sample list and set replicate number for unknowns to 3.
- 7. Perform "Needle desorb" after the Carryover Check if sample to sample carryover was indicated in the results.

#### Instruction: Gyrolab Carryover Check, D0014825/E, Page 2 of 4

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### Perform Carryover Check using Gyrolab Manager

- 1. Perform "Needle desorb" before running the Carryover Check.
- 2. Create a Run design, using the method specific for the immunoassay, with a standard series, blank and 7 standard points, in triplicate. Create an experiment and set "Unknown sample replicates" to 3.
- 3. Save the design and choose to create a Run setup from the Run Design.
- 4. Create a sample list:
  - a. Select File/Generate sample list template.
  - b. Set the number of samples to 24.
  - c. Choose to include all samples.
  - d. Save the sample list.
- 5. Open the generated sample list and edit the MP well positions according to below:

٠	Blank and standard points	B1-B8

- Unknown samples #1-8 (corresponds to blanks before) A1-A8
- Unknown samples #9-16 (corresponds to highest std sample) C1-C8
- Unknown samples #17-24 (corresponds to blanks after) D1-D8
- 6. Select File/Import sample list and select the generated sample list.
- 7. Save the Run Setup.
- 8. Prepare Carryover Check:
  - a. Dilute samples in the matrix and Rexxip buffer normally used.
  - b. Prepare the standard series, one blank and 7 standard series samples in different concentrations and place them in the microplate according to Table 1.
    - Use the highest standard sample concentration in the standard series for the immunoassay as the high concentration in the check.
    - The blank should contain the same matrix and Rexxip buffer as in the standard series.
- 9. Execute the Carryover Check Run setup using Gyrolab Control.
- 10. Perform "Needle desorb" after the Carryover Check if sample to sample carryover was indicated in the results.

### Analysis of results

Evaluate results in Gyrolab Evaluator. The concentration levels for the "blanks before" and "blanks after" the highest standard concentration should <u>not</u> be quantifiable.

Quantifiable concentration in the "blanks before" is an indication that the instrument is contaminated. Contact your Gyrolab Field Application Specialist for further support and for suggestion on alternative Needle desorb solution and method that is suitable to your analytical situation.

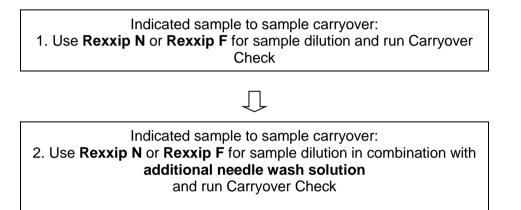
If the "blanks after" (i.e. blank sample run after the needles have been in contact with the high standard series concentration) shows quantifiable concentrations, sample to sample carryover under the specific assay conditions may be occurring. Parameters such as Rexxip buffer, needle wash solution and Gyrolab methods should be reviewed.

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### Actions to prevent sample to sample carryover

Make sure the recommended <u>Rexxip buffers and/or needle wash solutions</u> are used according to the properties of the analyte in the specific immunoassay. See figure 1 below.

- For guidance of recommended Rexxip buffer see "Instruction For Use: Rexxip buffer" (document number D0010353). The document is included in each Rexxip delivery.
- For guidance of recommended Rexxip buffer and additional needle wash solution see "Instruction: Gyrolab method including additional needle wash" (document number D0012293).



**Figure 1**. Workflow describing how to achieve optimal immunoassay performance in Gyrolab if sample to sample carryover was indicated.

For further questions and support, contact Gyrolab Field Application Specialist.

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