

# Galactokinase – Sample type and stability study

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## Introduction & Aim

Quantitation of Galactokinase activity is used to diagnose or exclude galactokinase deficiency (OMIM 604313), which is an important cause of bilateral juvenile cataracts. Early treatment with a galactose-restricted diet may prevent or reverse the formation of cataracts. Due to enzyme lability, laboratories have strict requirements for collection, transport and storage of clinical samples, although these differ between laboratories. The aim of this study was to investigate the stability of galactokinase in red blood cells under a wide range of different sample types and storage conditions.

## Room temperature 24–48 hr

Galactokinase activity was stable in both lithium heparin and EDTA samples at room temperature and 4°C for 24 hours. When analysed at 48 hours, samples that had been stored at room temperature since collection showed 17.4 - 20.4% loss of activity. In the same time-frame but stored at 4°C, Lithium heparin samples were stable but EDTA samples gave discrepant results.

	Room temp 24 hours		Room temp 48 hours	
	Activity	% Loss	Activity	% Loss
Li Hep	1.88	-1.0	1.52	-19.8
	1.87	-1.6	1.51	-20.4
EDTA	1.90	-0.2	1.57	-17.4
	1.87	-1.4		
	4°C 24 hours		4°C 48 hours	
	Activity	% Loss	Activity	% Loss
Li Hep	1.90	-0.2	1.84	-3.0
	1.87	-1.4	1.80	-5.4
EDTA	1.97	3.6	1.86	-2.0
	1.94	1.9	2.12	11.3

## Methods

An HPLC-MS/MS method adapted from Ko DH, et al Clin Chem 2010; 56(5): 764-71, was used to quantitate galactokinase activity in red blood cells.

Four Lithium heparin tubes and four EDTA tubes were filled with blood directly from one adult (day 0). Bloodspots were made (from a small portion) and then each tube stored at different temperatures for different amounts of time according to the table below, before the red blood cells (RBC) were washed with saline.

Storage of whole blood before washing red cells	
Lithium Heparin	EDTA
<2 hours	< 2 hours
Room temp: 1 day	Room temp: 1 day
Room temp: 2 days	Room temp: 2 days
4°C: 2 days	4°C 2 days
4°C: 4 days	

Each tube of washed cells was then split and several 50µL aliquots made. Aliquots were stored at either 4°C or -156°C until analysis. The assay was performed on days 1, 2, 4 and 8, with samples run in duplicate.

This study was part of initial assay validation. The assay is now UKAS accredited and has been in routine clinical use at NBT since 1/1/21, replacing the earlier radiometric C14 enzyme assay.

## Whole blood stored at 4°C

	Analysed Day 1			Analysed Day 4			Analysed Day 8		
	Day Cells washed	Activity	% Loss	Day Cells washed	Activity	% Loss	Day Cells washed	Activity	% Loss
LiHep	0	1.90	-0.2	0	1.69	-11.1	2	1.65	-13.0
	0	1.87	-1.4	0	1.74	-8.7	2	1.73	-9.1
				2	1.84	-3.0	4	1.83	-3.9
				2	1.80	-5.4	4	1.72	-9.6
	Analysed Day 1			Analysed Day 4					
	Day Cells washed	Activity	% Loss	Day Cells washed	Activity	% Loss			
EDTA	0	1.97	3.6	0	1.85	-2.5			
	0	1.94	1.9	0	2.13	12.2			
				2	1.86	-2.0			
				2	2.12	11.3			

When cells are washed on day 0 and stored at 4°C, galactokinase is stable in lithium heparin and EDTA samples for 24 hr,

but results are low / discrepant by day 4. When samples are stored as whole blood and cells washed closer to the assay date, stability is improved for lithium heparin samples but remain discrepant in EDTA samples.

On day 8 the aliquots which had been washed on day 4 showed better stability and reproducibility than those washed on day 2. As a result of this study, our processes have been revised so cells are now washed on the day of the assay, rather than on arrival in our laboratory.

## Other Storage Conditions

Storing washed cells at -156°C, gave low and discrepant results (data not shown). Bloodspots prepared on day 1, stored at room temperature, analysed on day 8, showed a loss of 83.3% - 90.4% galactokinase activity. Preparing a RBC lysate and storing at either 4°C or -156°C, analysed on day 4 showed loss of 9.0 - 100 % galactokinase activity.

## 24hr at Room temperature, then washed RBC stored at 4°C

In routine practice, many clinical samples may not be stored at 4°C immediately after venepuncture. These data show lithium heparin samples stored at room temperature for 1 day then washed and cells stored at 4°C, were stable when assayed 1 and 3 days after washing, but not 7 days later (8 days after collection), by which time results were low and discrepant showing activity loss of -8.0 and -22.8%.

Days rbc stored at 4°C	Activity	% Loss
1	1.98	4.4
1	1.94	2.1
3	1.83	-3.7
3	1.78	-6.5
7	1.47	-22.8
7	1.75	-8.0

## New Sample Requirements

- 0.5mL lithium heparin whole blood to be stored at 4°C and shipped with an ice pack (same day / overnight transport).
- Telephone when sample arrives in your lab as it must be analysed within 5 days of collection.
- Ensure no RBC transfusion in previous 4 months.

For further details and new reference ranges, see [www.severn-pathology.com](http://www.severn-pathology.com)

