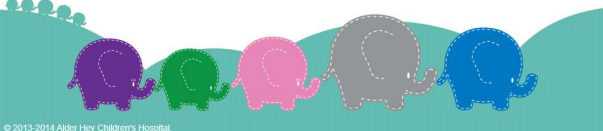
  
Alder Hey Children's  
NHS Foundation Trust

## Amino Acid Chromatography Troubleshooting

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## What's it all about?

- Amino Acid sample processing (in brief)
- What the Chromatography can tell us
- Pressure problems and what to do about them
- Understanding the program and how it can help us




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### Accelerated

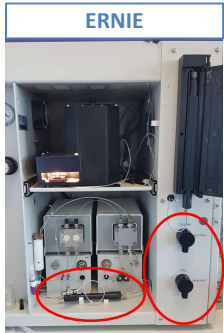
## Meet the analysers

**BERT**




BioChrom 30+ (H.P. program)

**ERNIE**




BioChrom 30 (Accel. program)



3


## Amino Acid Sample Processing



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## Processing samples (in brief)


- Plasma, CSF or urine acidified and deproteinised (1:1 with IS/SSA)
- Internal standard includes Norleucine + AEC
- 44 Aminos Acids measured over 120ish minutes
- Approx 2.5-3 hours total runtime per sample
- At Alder Hey we report 24 Amino Acids
- Operator checks every chromatogram



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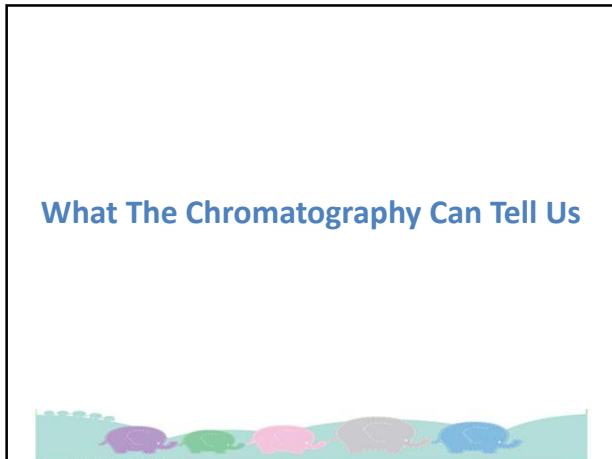
## Methodology

- Ion Exchange Chromatography
- Resin Column has -ve charged solid phase
- Acidified sample has +ve charged amino acids
- 6 mobile phase buffers of increasing pH and ionic strength
- Column temperature gradually increased
- Iso-ionic point reached – AA eluted off column
- Mixed with NIN and travels to detector

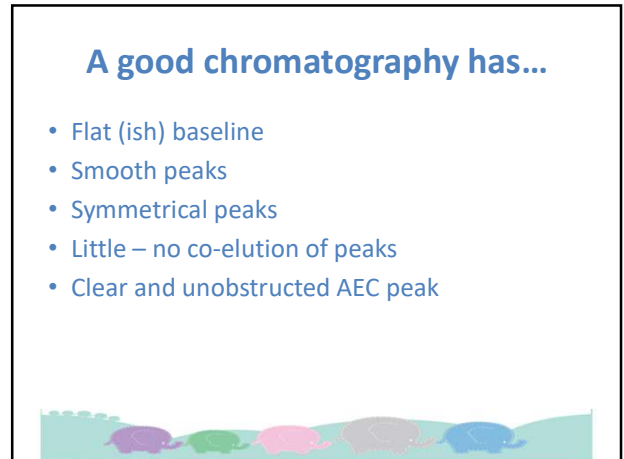


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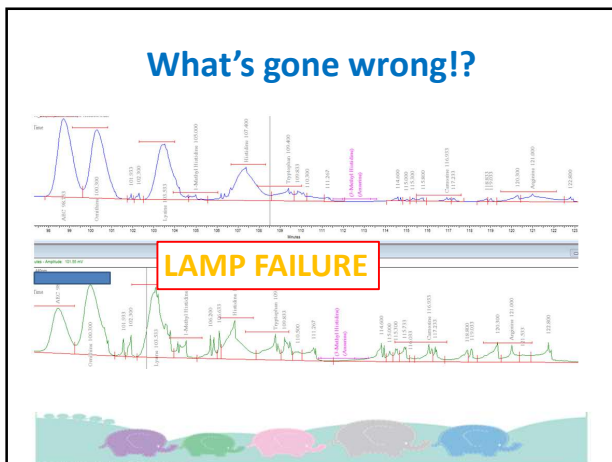




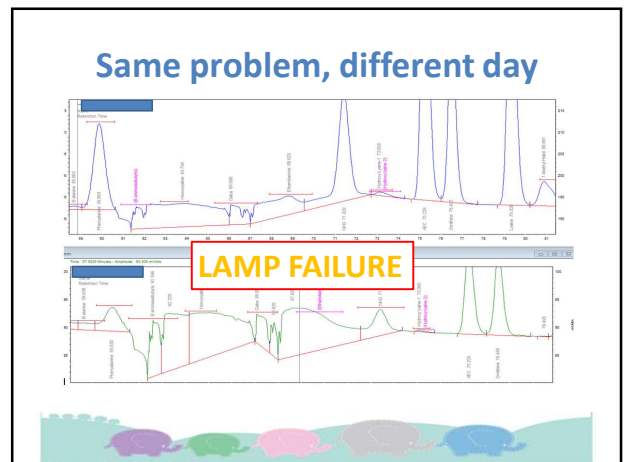
13



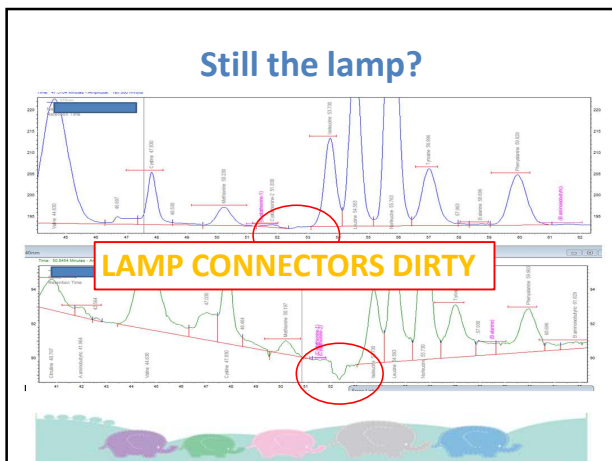
14



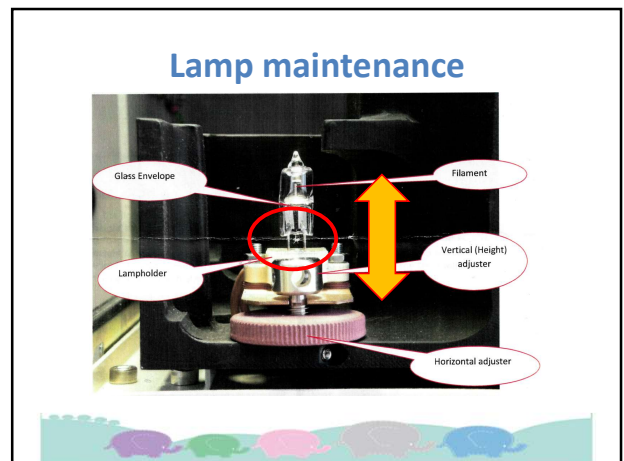
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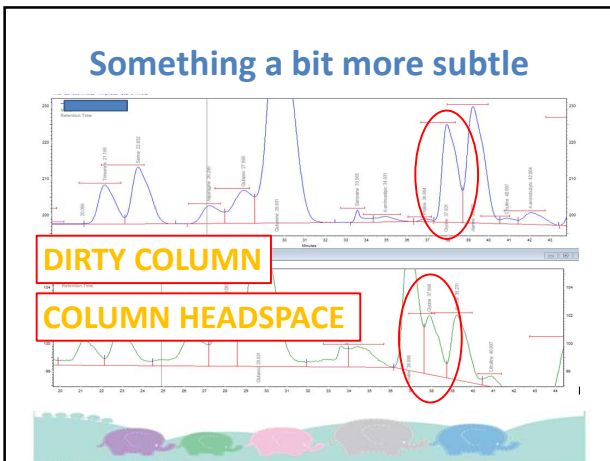


17



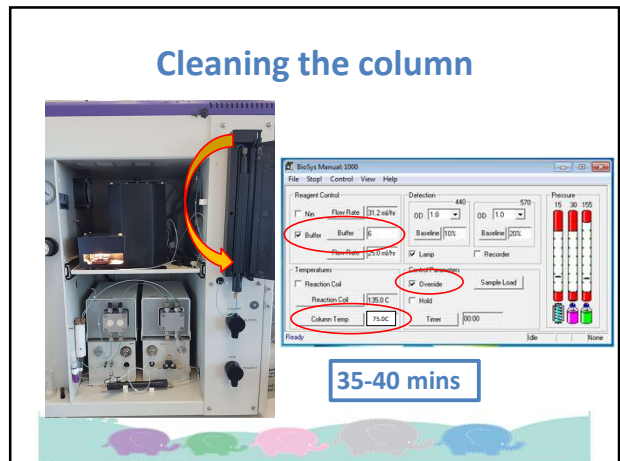
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### Something a bit more subtle



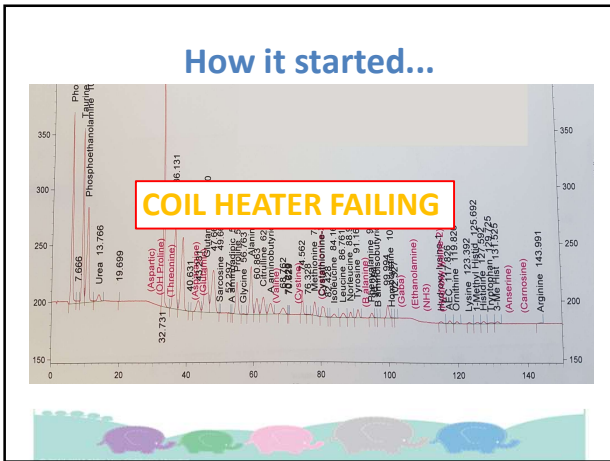
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### Cleaning the column



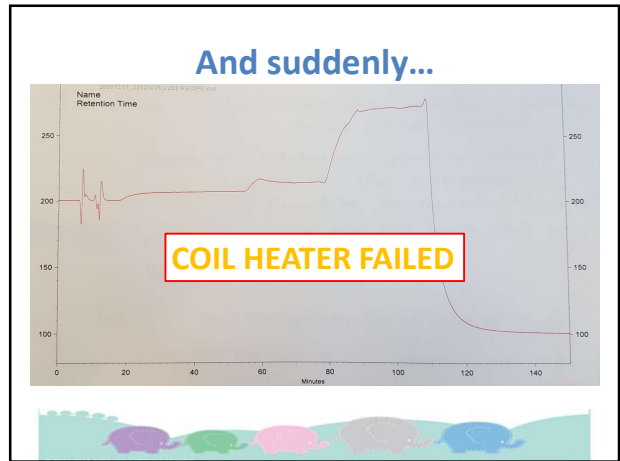
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### How it started...



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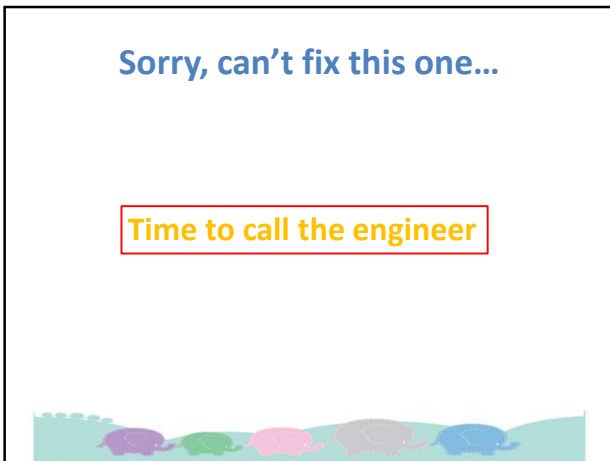
### And suddenly...



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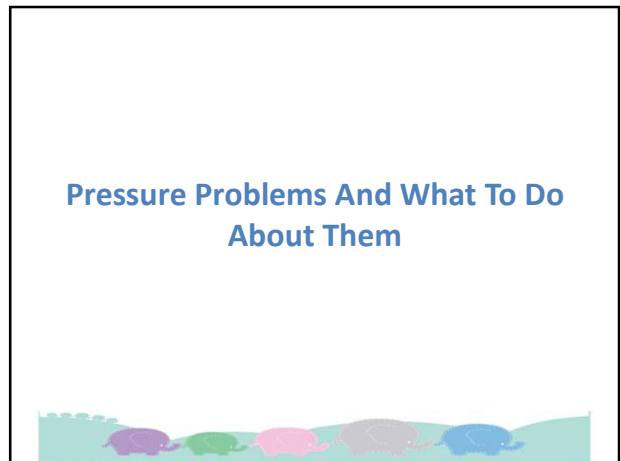
### Sorry, can't fix this one...

Time to call the engineer



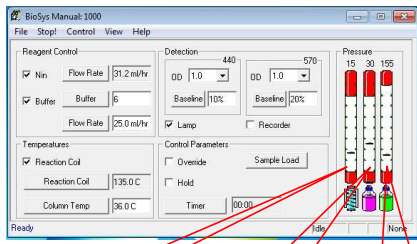
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### Pressure Problems And What To Do About Them



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### Pressure monitoring



Coil Pressure

NIN Pressure

Buffer Pressure

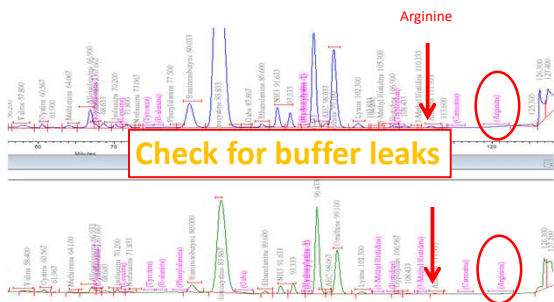
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### Part 1; Error message

NIN Pressure > Buffer Pressure

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### Part 2; Chromatography



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### Find a leak!



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### Now what?

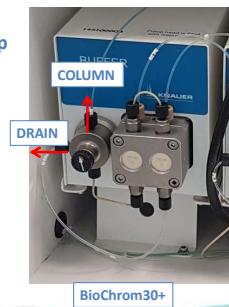


Check the ferrule

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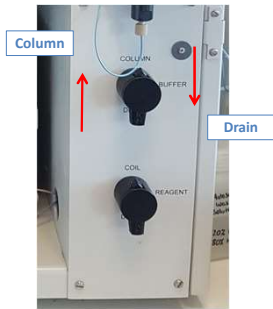
### No Leaks?

- Check all buffer are topped up (esp buffer 1 and 5)
- Bleed all lines to remove possible air bubbles (make sure to run to drain)



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### Or on the Biochrom 30



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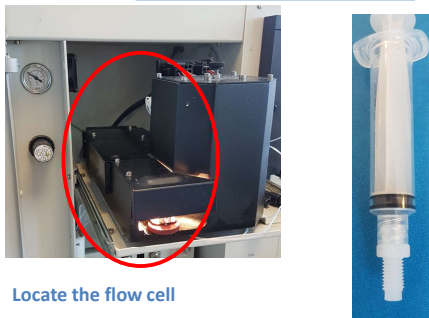
### Still no joy?

Could be the pump motor...

**Time to call the engineer**

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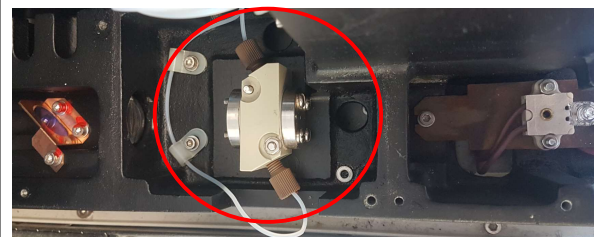
### Error: High Coil Pressure



Locate the flow cell

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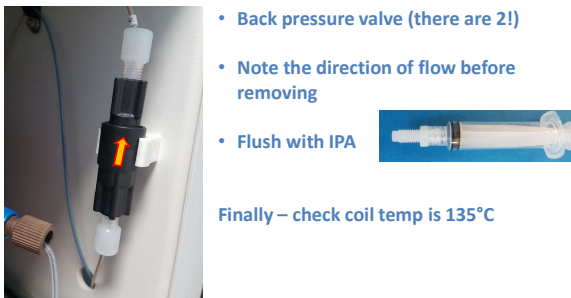
### Flow cell



Remove and flush with IPA

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### Error: High Coil Pressure

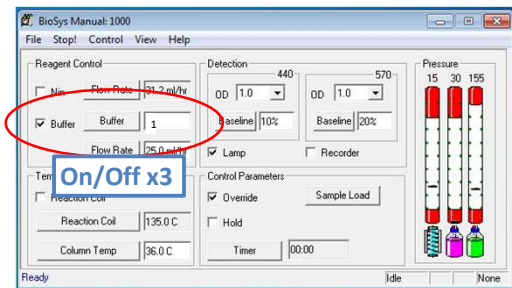


- Back pressure valve (there are 2!)
- Note the direction of flow before removing
- Flush with IPA

Finally – check coil temp is 135°C

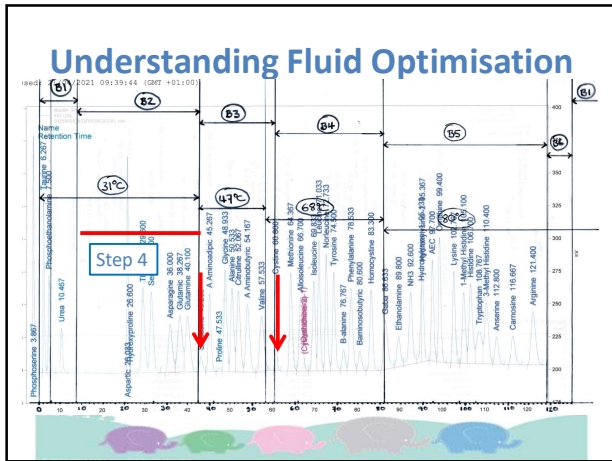
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### Error: Low Coil Pressure



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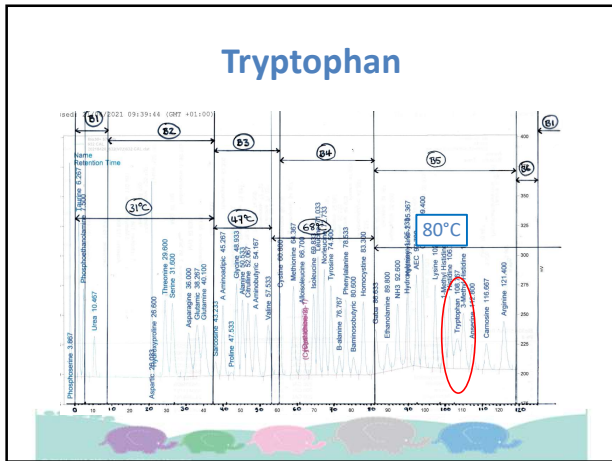
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### Program tweaks

Most common = Tryptophan

- Physiological Fluid Optimisation says "Decrease/Increase T3"??
- We actually want to look at what the temperature is where Tryp comes off

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### Tryptophan

No.	Time	Temp	Buffer	Pump	Nin	Rec	Commands
1	01:00	31°C	1	25.0ml/h	ON	OFF	
2	01:00	31°C	1	25.0ml/h	ON	OFF	Reset
3	01:00	31°C	1	25.0ml/h	ON	OFF	Load
4	02:00	31°C	1	25.0ml/h	ON	OFF	
5	03:00	31°C	1	25.0ml/h	ON	OFF	Reset
6	04:30	31°C	1	25.0ml/h	ON	ON	
7	29:00	31°C	2	25.0ml/h	ON	ON	
8	15:45	47°C	3	25.0ml/h	ON	ON	
9	02:00	68°C	3	25.0ml/h	ON	ON	
10	26:00	80°C	4	25.0ml/h	ON	ON	
11	26:00	80°C	5	25.0ml/h	ON	ON	
12	06:00	80°C	6	25.0ml/h	ON	ON	
13	06:00	80°C	1	25.0ml/h	ON	ON	
14	02:00	50°C	0	OFF	OFF	OFF	
15	34:00	50°C	1	25.0ml/h	ON	OFF	
16	02:00	31°C	1	25.0ml/h	ON	OFF	
17	04:00	31°C	1	25.0ml/h	ON	OFF	
End							

Steps 11, 12 & 13  
T3 = 3<sup>rd</sup> Temp CHANGE

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### We made it to the end!

- Thanks for listening, any questions?

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