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Development of an External Quality Assurance Programme for a point-of-care test for phosphorylated insulin-like growth factor binding protein-1(phIGFBP-1)

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Introduction

Pre-term labour is defined as regular contractions of the uterus resulting in changes to the cervix, starting before 37 weeks' gestation. Pre-term labour affects around 8 % of babies in the UK, and is associated with short-term health problems, including breathing and feeding; as well as long-term physical and learning disabilities.

Phosphorylated insulin-like growth factor binding protein-1 (phIGFBP-1) is produced in the decidua, and as the cervix matures with approaching labour, the decidua and chorion detach, allowing phIGFBP-1 to leak into cervical secretions. The Actim Partus (Alere, UK) is a qualitative point-of-care test (POCT) designed to detect phIGFBP-1 in cervical secretions during pregnancy, thus identifying women at risk of pre-term labour with intact membranes. This may aid clinicians in deciding whether a woman should be admitted to hospital for treatment to delay birth and improve neonatal outcomes.

In 2015, Weqas began development of an External Quality Assessment (EQA) programme for phIGFBP-1 to assess and monitor performance of these tests. The programme is now accredited to ISO 17043.

Materials

Semi-purified IGFBP-1 was sourced from Hytest Ltd., Finland, comprising extracts from amniotic fluid, purified by salt-fractionation and ion-exchange chromatography. Samples are diluted in phosphate buffered solution, containing bovine serum albumin, protease inhibitors and preservatives (Medix Biochemica, Finland).

Methods

The source material was validated using a quantitative immunoenzymometric assay in collaboration with Medix Biochemica, showing that phIGFBP-1 was present in the source material at a ratio of 1:2.4 (Figure 1)

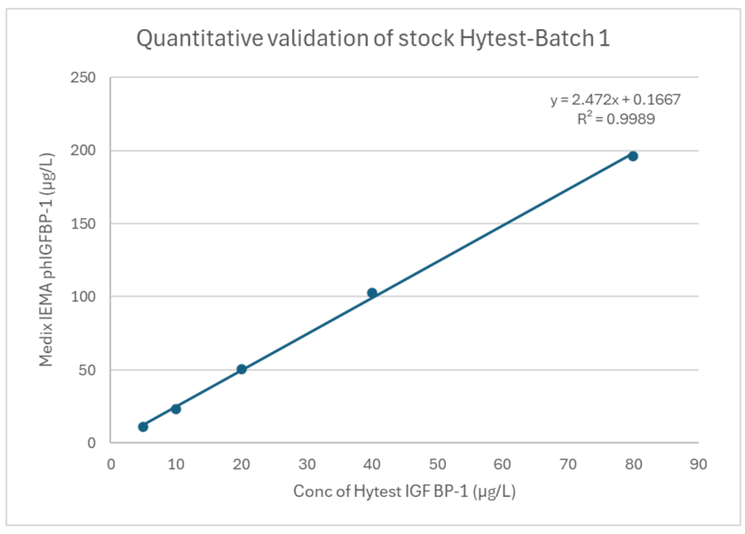

Figure 1: Quantitative validation of source material indicated a IGFBP1:phIGFBP-1 ratio of 1:2.4

EQA material comprising a range of concentrations was produced, including a true negative (diluent only); samples near the clinical cutoff (10 µg/L) and at the upper limit of detection (200 µg/L). Sample stability was assessed by storage of samples at 20 °C, 4 °C and -20 °C. Homogeneity was assessed by repeated analysis of multiple pools.

Results

Stability

Two pools with phIGFBP-1 values exceeding the positive cut-off limit of 10 µg/L were stored at 20 °C, 4 °C and -20 °C, and assayed over a period of 7-14 days. There was no significant deterioration in phIGFBP-1 over 14 days in samples stored at 4 °C and -20 °C; and no deterioration in phIGFBP-1 over 7 days in samples stored at 20 °C; with all results within expected variance for the method (Figure 2)

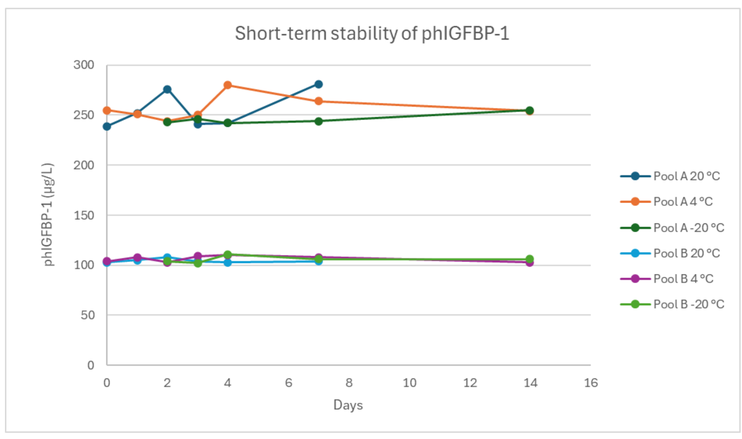

Figure 2: Short-term stability of phIGFBP-1 was assessed at 250 µg/L and 100 µg/L, at 20 °C, 4 °C and -20 °C, demonstrating acceptable stability for distribution

Three pools with phIGFBP-1 values at 80 µg/L, 40 µg/L and 20 µg/L were stored at -70 °C for 5 months and re-assayed. There was no significant change in phIGFBP-1 concentration in any of the pools, indicating long-term stability was acceptable (Figure 3)

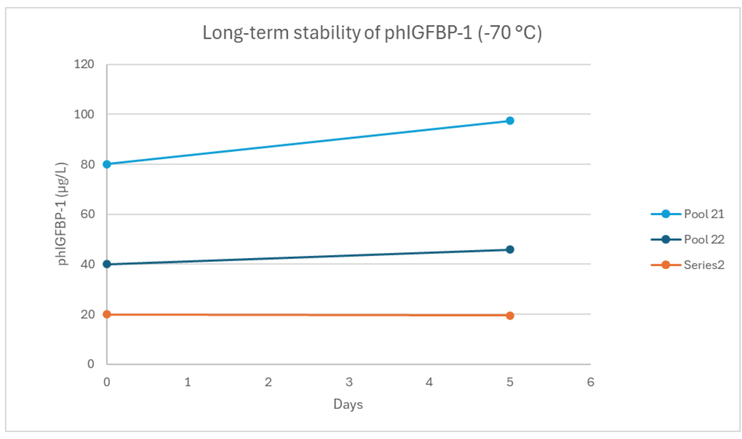

Figure 3: Long-term stability of phIGFBP-1 was assessed at 80 µg/L, 40 µg/L and 20 µg/L at -70 °C, demonstrating acceptable stability for long-term storage

Homogeneity

Following preparation of each pool, material was dispensed aseptically into aliquots and stored at -70 °C until dispatch. Homogeneity of the final product was assessed by analysis of multiple samples from each pool, calculation of within-sample standard deviation, and comparison to the Weqas standard deviation. Homogeneity was judged to be acceptable over multiple pools.

Analyte: phIGFBP-1			
Batch no: 010415/2	Pool code: 21		
	Result a	Result b	Sample Ave
Sample 1	95	101	98
Sample 2	96	96	96
Sample 3	103	99	101
Sample 4	94	94	94
Sample 5	106	95	100.5
Sample 6	95	94	94.5
Sample 7	95	104	99.5
Sample 8	89	88	88.5
Sample 9	103	101	102
Sample 10	98	102	100
General Average	97.400		
Sum d^2	276		
Within Sample SD (SD_{diff})	3.714835124		
SD of sample ave	4.169	Between sample SD < 0.3*Weqas SD?	
Between sample SD	3.23693957		
Weqas SD	19.48		
0.3*Weqas SD	5.844		ACCEPTED

Table 1: Example of in-house homogeneity calculations

Scheme design

Two samples are distributed to participants on a bimonthly schedule. Following preparation, material is dispensed aseptically into 1 mL aliquots and stored at -70 °C until dispatched. The samples are dispatched by first class mail packaged in containers conforming to Post Office guidelines. Although every effort is made to ensure that the material is free from any known infectious agent, the samples should be handled as for clinical specimens. Separate instructions are provided with each batch of material. Qualitative results are reported to Weqas via the online portal. Participant reports are produced according to standard Weqas processes, with IEMA quantitative results used to determine correct interpretation.

As part of continuing development of the programme, participant results are regularly reviewed to identify areas to focus on. As part of the pilot programme, performance data was reviewed, and showed that although clinical sensitivity exceeds 85 % at concentrations above 50 µg/L, it falls to 62 % at 12.5 µg/L, indicating negative results may be reported for a true positive sample at concentrations above the cut-off point. However, analysis of the data for more recent pools, distributed between June 2023 and April 2024 showed improved performance, achieving clinical sensitivity of 100 % at 25 µg/L, and exceeding 85 % at 12.5 µg/L.

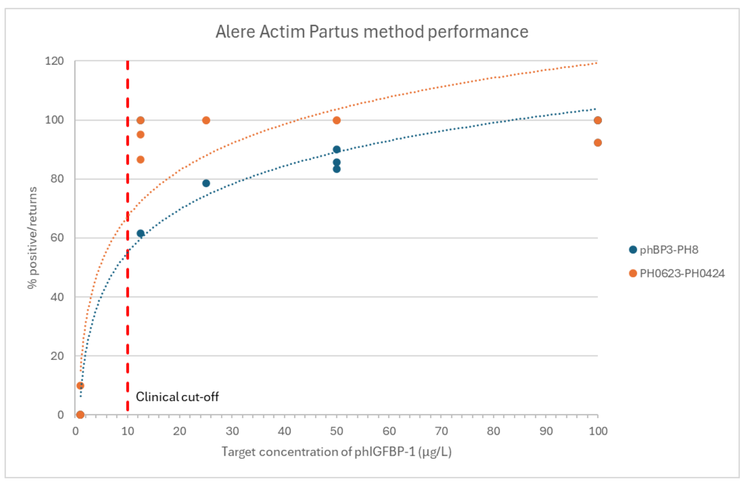

Figure 4: Performance data was analysed during the pilot programme (phBP3-BH8) and during the most recent distribution (PH0623-PH0424), demonstrating an improvement in performance, especially near the clinical cut-off

Conclusion

The Actim Partus (Alere, UK) is a qualitative point-of-care test (POCT) designed to detect phIGFBP-1 in cervical secretions during pregnancy, thus identifying women at risk of pre-term labour with intact membranes. An EQA programme assessing and monitoring performance of the Actim Partus test was designed and implemented. In-house stability and homogeneity experiments demonstrated that the material produced was suitable for distribution to participants. Assessment of performance data showed improvement in assay performance between initiation of the programme and the most recent distributions, especially near the clinical cut-off point. The data reiterates the need for an ongoing assessment of performance especially near the cut-off point. The Weqas Pre-Term Labour Marker programme is accredited to ISO 17043.