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# A novel cell-based assay for measuring neutralizing autoantibodies against type I interferons in patients with autoimmune polyendocrine syndrome type 1

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**Abstract** An important characteristic of autoimmune polyendocrine syndrome type 1 (APS 1) is the existence of neutralizing autoantibodies (nAbs) against the type I interferons (IFN) -α2 and -ω at frequencies close to 100%.
Type 1 IFN autoantibodies are detected by antiviral neutralizing assays (AVA), binding assays with radiolabelled antigens (RLBA), enzyme-linked immunosorbent assay (ELISA), or by reporter-based cell assays. We here present a simple and reliable version of the latter utilizing a commercially available cell line (HEK-Blue IFN-α/β).
All 67 APS 1 patients were positive for IFN-ω nAbs, while 90% were positive for IFN-α2 nAbs, a 100% and 96% correlation with RLBA, respectively. All blood donors and non-APS 1 patients were negative. The dilution titer required to reduce the effect of IFN-ω nAbs correlated with the RLBA index.
This cell-based autoantibody assay (CBAA) is easy to perform, suitable for high throughput, while providing high specificity and sensitivity.


## 1. Introduction

Autoimmune polyendocrine syndrome type I (APS 1) is a unique monogenic disorder caused by loss-of-function mutations in the Autoimmune Regulator (*AIRE*) gene [1,2]. AIRE is expressed in the thymus where it promotes transcription of organ specific

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genes allowing for the negative selection of pathogenic autoreactive T cells that otherwise would be allowed to move into peripheral tissues [3].

A wide range of autoimmune manifestations occur in APS 1, but the most prevalent clinical manifestations are chronic mucocutaneous candidiasis (CMC), hypoparathyroidism (HPT) and primary adrenocortical failure (autoimmune Addison's disease—AAD) [4]. The clinical criteria for diagnosis of APS 1 are two or more (one if a sibling has APS 1) of the above mentioned manifestations and/or disease-causing mutations in *AIRE* [4]. The time frame of the evolution of the disease is highly variable and it can take several years before the diagnosis can be made by clinical criteria; hence, the diagnosis may be delayed or missed [4–6].

APS 1 patients develop autoantibodies against proteins of the affected organs [7], such as 21-hydroxylase (adrenal gland) [8], side-chain cleavage enzyme (SCC) (adrenal cortex and ovaries) [8] and NACHT, LRR and PYD domain-containing protein 5 (NALP5) (parathyroid gland and ovaries) [9]. Although these antibodies can be used as diagnostic markers of the various components of APS 1, they are not present in all patients, nor are they necessarily specific for this disease [7]. In addition to the organ specific autoantibodies, APS 1 patients also have high titers of circulating neutralizing autoantibodies (nAbs) against several cytokines, such as several type I interferon isoforms (in particular IFN-α2 and -ω) [10,11] and IL-17/-22 [12,13]. Serum IFN-ω autoantibodies are being implemented as an important diagnostic tool, complementing genetic mutational analysis of *AIRE*, as they are found in close to all APS 1 patients before manifestations of most symptoms [14,15], in one case even prior to any other symptoms [15]. IFN-ω nAbs have also been observed in late onset myasthenia gravis and thymoma [16]. Several assays are currently available for detecting IFN-ω antibodies including immunoassays [10,17], radioligand binding assay (RLBA) [11] and the antiviral neutralization assay (AVA) [10]; however, these can be less sensitive, time-consuming, require facilities for growing virus cultures, or use radioactivity.

The objective of this study was to create a simple, inexpensive, and robust assay for detecting type I interferon neutralizing autoantibodies (IFN-ω and -α2) suitable for high throughput and where all reagents are commercially available.

## 2. Methods

### 2.1. Patients

Sera from a total of 67 APS 1 patients were studied, 32 from the Norwegian register for organ-specific autoimmune diseases (ROAS) [18], and 35 from a Russian APS 1 cohort [19]. RLBA data presented in this paper are either historical data from the ROAS register (Norwegian patients) or unpublished data (Russian patients). RLBA was performed as described earlier [11]. All Norwegian and Russian APS 1 patients were IFN-ω nAb positive as confirmed by RLBA tests in the Bergen lab. For IFN-α2 nAbs, we have data for 24/32 ROAS patients and 35/35 Russian patients. As further controls, we included 19 samples (10 for IFN-α2) from patients without APS 1 (both Norwegian and Russians), who were either relatives of patients with APS-1 or patients with other endocrine autoimmune diseases (APS 2 or AAD). All patients had signed informed consent according to the Helsinki declarations. In addition 123 anonymous healthy blood donors were recruited from Haukeland University Hospital Blood Bank as controls.

### 2.2. HEK-Blue cell maintenance

HEK-Blue IFN-α/β cells (Invivogen) were maintained in DMEM cell medium (Life Technologies) (4.5 g/l glucose, 4 mM L-alanyl-L-glutamine and 1 mM sodium pyruvate) supplemented with 10% (*v*/*v*) fetal bovine serum, 50 U/ml streptomycin, 100 μg/ml normocin, 30 μg/ml blasticidin and 100 μg/ml zeocin. Cells were cultured at 37 °C, 5% $O_2$ and passaged at 70–80% confluency and maintained for no longer than 3 weeks to ensure genetic stability. Cells were counted with the Countess Automated Cell Counter (Life Technologies).

### 2.3. IFN dose—response

Dose–response curves were created by serial two-fold dilution (2×) for both the type I IFNs for IFN-α (25–200 U/ml) (PBL Interferon Source), for IFN-ω (1.25–4 ng/ml) (PBL Interferon Source) and the type II IFN-γ 63–1000 ng/ml (Biolegend).

### 2.4. Cell-based autoantibody assay

Fig. 1 illustrates the general scheme of the cell-based autoantibody assay (CBAA). 6 μl patient serum was preincubated together with the appropriate type I IFN and diluted with PBS to a total volume of 60 μl for 30 min at RT. 20 μl of the preincubated solution (final serum dilution 1:100) was then added to a well of a flat bottom 96 well cell-culture plate together with approximately 50 000 HEK-Blue IFN-α/β cells in suspension; each sample was run in duplicate. The plate was then incubated for 21 h at 37 °C, 5% $O_2$ to allow for expression and secretion of IFN controlled secretable alkaline phosphatase (SEAP).

The chosen incubation time was a result of real time monitoring of IFN response where the standard cell experiment medium was replaced with HEK-Blue detection medium (Invivogen). Overnight incubation was required to separate the positive from the negative serum pool for all cell densities tested (data not shown).

### 2.5. Quantification of the cell-based activity assay

20 μl cell supernatant was incubated with 180 μl QUANTI-Blue (Invivogen) in a well of a flat bottom 96 well plate for 2 h at 37 °C, 5% $O_2$. The amount of secreted alkaline phosphatase was measured by a spectrophotometer at 650 nm.

Pooled blood donor sera were used as the negative control and the positive control was a pool of 20 patients positive for both IFN-α and -ω antibodies. IFN inhibition is presented as a percentage of neutralization [($Abs_{650}$ negative − $Abs_{650}$ sample / $Abs_{650}$ negative − $Abs_{650}$ positive) × 100]. The threshold for a positive sample, the upper normal limit, was set the mean of blood donors + 3 × standard deviations, i.e. 13 (ω) and 9 (α2) (mean ± SD ω: −2 ± 5, α2: −1.0 ± 3.2) (Fig. 3A and B).